

DOKUZ EYLUL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED
SCIENCES

SYNTHESIS, CHARACTERIZATION AND
ANTIMICROBIAL ACTIVITIES OF
BORON-STARCH COMPLEXES

by

Elif ANT BURSALI

December, 2010

İZMİR

**SYNTHESIS, CHARACTERIZATION AND
ANTIMICROBIAL ACTIVITIES OF
BORON-STARCH COMPLEXES**

**A Thesis Submitted to the
Graduate School of Natural and Applied Sciences of Dokuz Eylul University
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Philosophy in Chemistry, Chemistry Program**

**by
Elif ANT BURSALI**

December, 2010

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Ph. D. THESIS EXAMINATION RESULT FORM

We have read the thesis entitled “**SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITIES OF BORON-STARCH COMPLEXES**” completed by **ELİF ANT BURSALI** under supervision of **PROF. DR. MÜRÜVVET YURDAKOÇ** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Doctor of Philosophy.

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Elif ANT BURSALI

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITIES OF BORON-STARCH COMPLEXES

ABSTRACT

Boron complexes of starch and starch/poly (vinyl alcohol) (PVA) hydrogels, were synthesized with or without using glutaraldehyde (GA), epichlorohydrin (EPI) and N-(aminoethyl)-aminopropyl-trimethoxysilane (Z-6020) as cross-linking agents. The obtained complexes were characterized by using Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), scanning electron microscope (SEM) and X-ray diffraction analysis (XRD) methods. Degree of solubility, swelling and tensile strength tests were performed for the synthesized complexes. The antimicrobial activities of prepared boron-starch complexes either in powder or hydrogel form were assayed with in vitro conditions by the disc diffusion susceptibility tests for five different bacterial cultures and one fungus. Standard disks of amoxicillin/clavulanic acid (AMC), ofloxacin (OFX), netilmycin (NET), erythromycin (ER) and amphotericin B (AFB) were individually used as positive controls during antimicrobial activity testes. Inhibition zone formations confirmed that, starch/PVA hydrogel cross-linked with GA and boron complex of this hydrogel showed moderate antimicrobial activity against tested microorganisms, the latter being more efficient and more pronounced on Gram-negative than on Gram-positive bacteria. Other hydrogels and powder complexes had no antimicrobial activities against tested microorganisms. Considering all results, it could be claimed that the boron complexes of starch/PVA hydrogels cross-linked with GA might be used for biomedical applications.

Keywords: Starch, boron, hydrogels, cross-linking, antimicrobial activity

BOR-NİŞASTA KOMPLEKSLERİNİN SENTEZİ, KARAKTERİZASYONU VE ANTİMİKROBİYAL AKTİVİTELERİ

ÖZ

Nişasta ve nişasta/poli (vinil alkol) (PVA) hidrojellerinin bor kompleksleri; çarpaz bağlayıcı olarak glutaraldehit (GA), epiklorhidrin (EPI) ve N-(aminoetil)-aminopropil-trimetoksisilan (Z-6020) kullanarak ya da kullanmadan sentezlenmiştir. Elde edilen kompleksler Fourier dönüşümlü kızılötesi spektroskopisi (FTIR), termogravimetrik analiz (TGA), taramalı elektron mikroskobu (SEM) ve X-ışınları kırınım analizi yöntemleri kullanılarak karakterize edilmiştir. Sentezlenen kompleksler için çözünürlük, şişme derecesi ve çekme dayanımı testleri gerçekleştirilmiştir. Sentezlenen gerek toz gerekse hidrojel yapısındaki bor-nişasta komplekslerinin antimikrobiyal aktiviteleri in vitro koşullarda disk difüzyon duyarlılık testleri kullanılarak beş farklı bakteri ve bir mantar kültürü için tayin edilmiştir. Antimikrobiyal aktivite testleri sırasında; Amoksisilin/Klavulonik asit (AMC), Ofloksasin (OFX), Netilmisin (NET), Eritromisin (ER) ve Amfoterisin B (AFB) pozitif kontroller olarak ayrı ayrı kullanılmıştır. İnhibisyon bölgesi oluşumu GA ile çarpaz bağlanmış nişasta/PVA hidrojelini ve bu hidrojinin bor kompleksinin test edilen mikroorganizmalara karşı yeterli antimikrobiyal aktivite gösterdiklerini ki sonrakinin daha etkili olduğunu ve Gram-negatiften çok Gram-pozitif bakteri üzerine yoğunlaştığını doğrulamıştır. Diğer hidrojellerin ve toz komplekslerin test edilen mikroorganizmalara karşı antimikrobiyal aktivitesi bulunmamaktadır. Tüm sonuçlar değerlendirildiğinde, GA ile çarpaz bağlanmış bor-nişasta/PVA kompleksinin biyomedikal uygulamalarda kullanılabileceği söylenebilir.

Anahtar sözcükler: Nişasta, bor, hidrojeller, çarpaz bağlama, antimikrobiyal aktivite

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CHAPTER ONE

INTRODUCTION

1.1 Starch

Starch is a renewable, biodegradable and relatively inexpensive carbohydrate biopolymer derived from abundant and readily available sources (Ke & Sun, 2003, Xing, Zhang, Ju & Yang, 2006). The term starch is used to describe a biopolymer system comprising predominantly of two polysaccharides - amylose (normally 20-30%) and amylopectin (normally 70-80%) (Yoshimura, T., Yoshimura, R., Seki & Fujioka, 2006). The two polysaccharides are made of glucose monomers as seen from Figure 1.1. Amylose is almost a linear polymer with α -D-(1/4) glycosidic linkages, while amylopectin is a highly branched polymer which also contains α -D-(1/6) glycosidic linkages at the branching points in addition to α -D-(1/4) glycosidic linkages (Karim, Norziah & Seow, 2000).

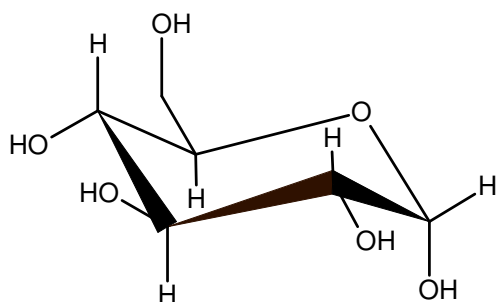


Figure 1.1 A glucose molecule, the basic building block of starch

The relative proportions of amylose to amylopectin and α -(1/6)- branch-points both depend on the source of the starch. Corn (maize), rice, wheat and potato are the main sources of starches which differ significantly in composition, morphology, thermal, rheological and retrogradation properties (Singh, N., Singh, J., Kaur, Sodhi & Gill, 2003).

The average granule size varies from source to source; rice starch granules are roughly 3 μm in diameter, corn starch has an average granule size of 10 μm , whereas potato starch granules are about 35 μm in diameter (Ramaraj, 2007).

1.1.1 Amylose

The smaller of the two polysaccharides which make up starch, amylose (Figure 1.2) is a linear molecule comprising of (1-4) linked α -D-glucopyranosyl unit. But it is today well established that some molecules are slightly branched by (1-6) alpha linkages (Buleon, Colonna, Planchot & Ball, 1998).

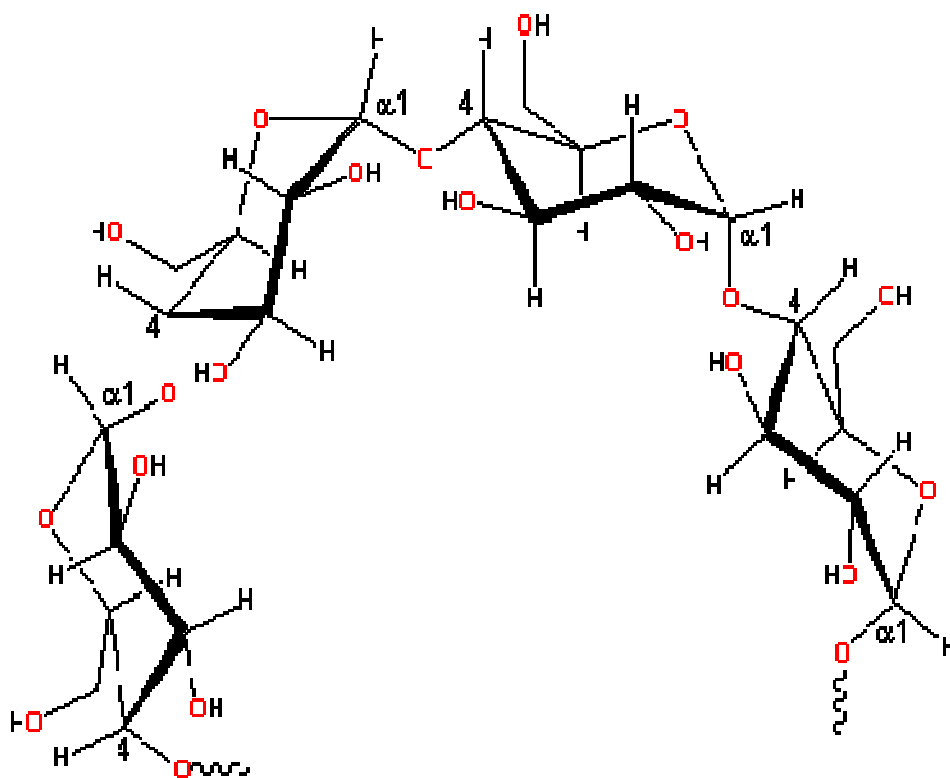


Figure 1.2 Representative partial structure of amylose

1.1.2 Amylopectin

The larger of the two components, amylopectin (Figure 1.3) is highly branched with a much greater molecular weight.

This structure contains alpha-D-glucopyranosyl units linked mainly by (1-4) linkages (as amylose) but with a greater proportion of (1-6) linkages, which gives a large highly branched structure (Yoshimura, Yoshimura, Seki & Fujioka, 2006).

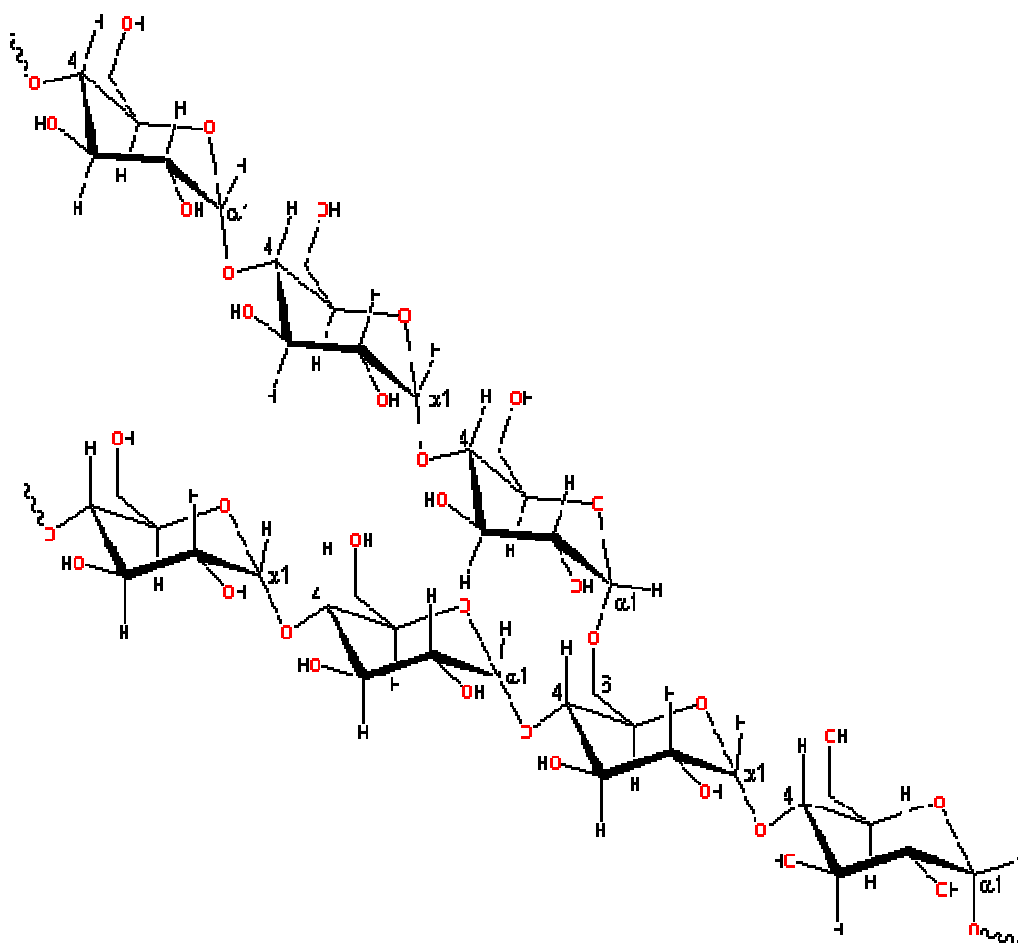


Figure 1.3 Representative partial structure of amylopectin

Amylopectin has been found to form the basis of the structure of starch granules. This is because the short branched (1-4) chains are able to form helical structures which crystallize.

1.1.3 Cross-linked Starch

The starch polymers are packaged by nature in the form of granules. Through hydrogen bonding, the amylose and amylopectin polymers form highly ordered crystalline bundles. These starch particulates are insoluble in water at ambient temperatures. However, as the water temperature is raised, the granules swell and rupture as the hydrogen bonds are broken. Starch undergoes two basic changes in its properties during heating and cooling; gelatinization and retrogradation (Raina, Singh, Bawa & Saxena, 2006).

Gelatinization is attributed to the diffusion of amylose outside the granule. Beyond a critical temperature (60 °C), the swollen starch granules can undergo a disruption into smaller aggregates or particles and result in a gelatinized starch (Cyras, Zenklusen & Vazquez, 2006). The performance properties of starches can be altered through such chemical modifications as cross-linking and hydrophobic substitution (Raina et al., 2006).

Chemically modified starches with improved properties are gaining increasing importance in industry not only because they are low in cost, but mainly because the polysaccharide portion of the product is biodegradable (Ebihara, Nakaï & Kishida, 2006, Janarthanan, Yunus & Ahmad, 2003).

Chemical cross-linking is a highly versatile method to create and modify polymers, where properties can be improved, such as mechanical, thermal and chemical stability. Cross-linking agents enhances not only the thermal properties but also the mechanical properties of blends by reinforcing the intermolecular binding with the introduction of covalent bonds to supplement natural intermolecular hydrogen bonds (Sreedhar, Chattopadhyay, Karunakar & Sastry, 2006).

Cross-linked starch is a starch that has been treated with one or more of multifunctional reagents capable of forming either ether or ester inter-molecular linkages at random locations between glucose residues in adjacent starch chains.

It is a derivative bridged more than two hydroxyl groups with multi functional groups. The cross linking can occur between molecules in a solution or in the starch grain (Acquarone & Rao, 2003, Wurzburg, 1986). When the specific reagent contains two or more moieties capable of reacting with hydroxyl groups, there is the possibility of reacting at two different hydroxyls resulting in cross-linking between hydroxyls on the same molecule or between hydroxyls on different molecules (Miyazaki, Hung, Maeda & Morita, 2006).

Cross-linking treatment is intended to add intra- and inter-molecular bonds at random locations in the starch granule that stabilize and strengthen the granule (Acquarone & Rao, 2003). Cross-linking reinforces the starch granule to be more resistant toward acidic medium, heat and shearing, and thereby decreased the solubility, swelling power and hence viscosity of modified starch from that of native starch (Yeh, A.I. & Yeh, S.L., 1993). Decrease in retrogradation rate and increase in gelatinization temperature have been observed with cross-linked starches, and these phenomena are related to the reduced mobility of amorphous chains in the starch granule as a result of the intermolecular bridges (Chung, Woo & Lim, 2004).

Even a very few cross-links (in the case of diesters) can drastically alter the paste and gel properties of the starch (Singh, Kaur & McCarthy, 2007). The cross-linking between two different hydroxyl groups of amylose and/or amylopectin molecules as activity of the specific reagent causes the granules to become compact and absorb less water than the native starch.

Cross-linking reinforces the hydrogen bonds in the granule with chemical bonds which act as a bridge between molecules. As a result, when the cross-linked starch is heated in water, the granule may swell as the hydrogen bonds are weakened; however, the chemically bonded cross-links may provide sufficient granule integrity to keep the swollen granules intact and minimize or prevent loss in viscosity (Miyazaki et al., 2006)

The type of cross-linking agent greatly determines the change in functional properties of the treated starches (Singh et al., 2007). The chemical and physical properties achieved when cross-linking starch depend on, for example, the chemical nature of the reagent, the degree of substitution, source of starch, reagent concentration, pH, reaction time and temperature (Hirsch & Kokini, 2002, Garg & Jana, 2007)

EPI, sodium trimetaphosphate, monosodium phosphate, sodium tripolyphosphate, phosphoryl chloride, a mixture of adipic acid and acetic anhydride, vinyl chloride, 2,3-dibromopropanol, acrylic acid, linear dicarboxylic anhydrides and a mixture of succinic anhydride-vinyl acetate are the main agents used to cross-link starches (Code of Federal Regulations [CFR], 1995, Miyazaki et al., 2006, Wattanchant, Muhammad, Hashim & Rahman, 2003).

1.1.3.1 Extent of Chemical Modification

The rate and efficiency of the chemical modification process depends on the reagent type, botanical origin of the starch and on the size and structure of its granules (Huber & BeMiller, 2001). This also includes the surface structure of the starch granules, which encompasses the outer and inner surface, depending on the pores and channels, leads to the development of the so-called specific surface (Juszczak, 2003).

Channels that open to the granule exterior provide a much larger surface area accessible by chemical reagents, and provide easier access by the reagents to the granule interior. However, the reagent may diffuse through the external surface to granule matrix in the absence of channels (BeMiller, 1997). Although starches from various sources exhibit fundamental structural similarities, they differ in the specific details of their micro structure and ultra structure. These structural differences have the potential to affect the chemical modification process (Huber & BeMiller, 2001).

The reactivity and concentration of reagents have been reported to influence the degree of substitution of cross-linked starches. Also, the type of reagent used and the reaction conditions determine the ratio of mono- and di-type bonds (esters with phosphorus based agents, and glycerols with EPI) during cross-linking (Koch, Bommer & Koppers, 1982).

Knowledge about the structural changes in starch granules caused by modification with chemical reagents can be of importance for understanding the altered functional properties, and for developing chemically modified starches with desired properties (Kim, Hermansson & Eriksson, 1992). Shiftan et. al. (2000) reported that EPI cross-linking is not homogeneous and is concentrated in the non crystalline domain of starch granules. Jane, Radosavljevic & Seib (1992) found that cross-linking of starch chains occurred mainly in amylopectin. Another factor that may influence the extent of cross-linking is the size distribution of starch granule population (Hung & Morita, 2005). During cross-linking small size granules have been reported to be derivatized to a greater extent than the large size granules (Bertolini, Souza, Nelson & Huber, 2003).

1.1.3.2 Physico-Chemical Properties

The physico-chemical properties of starches such as swelling, solubility, and light transmittance have been reported to be affected significantly by chemical modification. The change in these properties upon modification depends on the type of chemical modification.

Chemical modifications such as acetylation and hydroxypropylation increase, while cross-linking has been observed to decrease (depending on the type of cross-linking agent and degree of cross-linking) the swelling power and solubility of starches from various sources (Singh et al., 2007).

Choi and Kerr (2004) reported that the cross-linked starch granules have higher resistance towards temperature and heating time. Cross-linking strengthens the bonding between the starch chains, causing an increase in the resistance of the granules to swelling with increasing degree of cross-linking. Higher concentrations of fast acting cross-linking reagents such as POCl_3 result in greater reductions in the swelling potential as compared with slower acting agents such as EPI (Hirsch & Kokini, 2002).

Inagaki and Seib (1992) also reported that the swelling power of cross-linked waxy barley starch declined as the level of cross-linking increased. Cross-linked starches exhibit lower solubility than their native equivalents, and solubility decreases further with an increase in the concentration of cross-linking reagent, which may be attributed to an increase in cross-link density (Kaur, Singh, J. & Singh, N., 2006). Cross linking at low levels, although having a substantial effect on starch properties such as granule swelling contributes little to water sorption properties (Inagaki & Seib, 1992).

1.1.3.3 Morphological Properties

The starch granule itself is not structurally homogeneous from a physical and chemical point of view, since it has different physical natures (amorphous and crystalline regions) as well as different chemical compositions in each region (French, 1984).

After being cross-linked using EPI and POCl_3 , potato starch granules remain smooth and similar to native starch granules in morphology when viewed under SEM, suggesting that the modification does not cause any detectable morphological change (Kaur et al., 2006).

1.1.3.4 Thermal Properties

Starch gelatinization is the collapse (disruption) of molecular orders within the starch granule manifested in irreversible changes in properties such as granular swelling, native crystalline melting, loss of birefringence, and starch solubility (Atwell, Hood, Lineback, Varriano-Marston & Zobel, 1988).

Cross-linking alters the thermal transition characteristics of starch, the effect depending on the concentration and type of cross-linking reagent, reaction conditions and the botanical source of the starch. An increase in gelatinization temperature has been observed for cross-linked starches; these phenomena are related to the reduced mobility of amorphous chains in the starch granule as a result of the formation of intermolecular bridges (Singh et al., 2007).

Choi and Kerr (2004) reported that cross-linked starches prepared using a relatively low concentration of the POCl_3 had gelatinization parameters similar to those of native starches, while cross-linked starches prepared using higher reagent concentrations showed considerably higher T_c and ΔH_{gel} values. Type and concentration of the reagent; amylose/amylopectin ratio of starch during cross-linking significantly affects the extent of change in thermal properties (Singh et al., 2007).

1.1.3.5 Rheological Properties

Rheological properties of a material reflect its structure. During gelatinization, starch granules swell to several times their initial volume (Singh et al., 2007). Rheological behavior of starch is governed by amylose content, granule size distribution, granule volume fraction, granule shape, granule-granule interaction and continuous phase viscosity (Kaur et al., 2006).

Chemical modification leads to a considerable change in the rheological properties of starches. Modification method, reaction conditions and starch source are the critical factors that govern the rheological behavior of starch (Yeh, A.I. & Yeh, S.L., 1993).

Cross-linking has been reported to increase the shear stability, viscosity and pasting temperature of waxy rice starch, and to decrease pasting temperature of normal rice starch (Liu, Ramsden & Corke, 1999). Cross-linking leads to a higher increase in the peak viscosity of waxy starches as more amylopectin than amylose molecules have been reported to become cross-linked (Liu et al., 1999).

Cross-linking leads to an increase in the peak viscosity of both normal and waxy starches. Strengthening bonding between starch chains by cross-linking will increase the resistance of the granule towards swelling, leading to lower paste viscosity, which suggests that the concentration of the cross-linking reagent affects the structure within the granule, perhaps by affecting the distribution of the introduced cross-links. Cross-link location has also been reported to have varied effects on different properties of cross-linked starches (Yoneya, Ishibashi, Hironaka & Yamamoto, 2003).

Therefore, by appropriate choice of the native starch source (potato, maize, wheat etc.) and of the type of chemical modification and concentration of the modifying reagent, modified starches with very useful rheological properties can be obtained (Kaur et al., 2006).

1.2 Boron

Boron is a chemical element in the periodic table that has the symbol “B” and has an atomic number 5 (Figure 1.4). A trivalent metalloid element, boron has two naturally-occurring and stable isotopes, ^{11}B (80.1%) and ^{10}B (19.9%) (Parks & Edwards, 2005). There are several allotropes of boron; amorphous boron is a brown powder, but metallic boron is black. Boron is a semi-metallic element, exhibiting some properties of a metal and some of a non-metal.



Figure 1.4 Structure of Boron

It is a relatively rare element in the earth's crust, representing only 0.001%. Boron does not appear in nature in elemental form and always occurs in nature bound to oxygen in the form of borates. So boron is found combined in borax, boric acid, colemanite, kernite, ulexite and borates etc. (Table 1.1) (Parks & Edwards, 2005).

Boric acid is sometimes found in volcanic spring waters. Borate deposits are rare, being found in dry regions of the world such as the USA, Turkey, China and Russia (Jiang, Xu, Simon, Quill & Shettle, 2006).

Boron is widely distributed in surface and ground waters, occurring naturally or from anthropogenic contamination, mainly in the form of boric acid or borate salts (Sabarudin, Oshita, Oshima & Motomizu, 2005).

Table 1.1 Boron-Containing Minerals of Commercial Importance

Mineral	Chemical Composition	Boron (%)
Boracite	$\text{Mg}_6\text{B}_{14}\text{O}_{26}\text{Cl}_2$	19.30
Colemanite	$\text{Ca}_2\text{B}_6\text{O}_{11}\cdot 5\text{H}_2\text{O}$	15.78
Datolite	$\text{CaBSiO}_4\cdot\text{OH}$	6.76
Hydroboracite	$\text{CaMgB}_6\text{O}_{11}\cdot 6\text{H}_2\text{O}$	15.69
Kernite	$\text{Na}_2\text{B}_4\text{O}_7\cdot 4\text{H}_2\text{O}$	14.90
Priceite	$\text{Ca}_4\text{B}_{10}\text{O}_{19}\cdot 7\text{H}_2\text{O}$	15.48
Proberite	$\text{NaCaB}_5\text{O}_9\cdot 5\text{H}_2\text{O}$	15.39
Sassolite	H_3BO_3	17.48
Szaibelyite	$\text{MgBO}_2\cdot\text{OH}$	12.85
Tincal (borax)	$\text{Na}_2\text{B}_4\text{O}_7\cdot 10\text{H}_2\text{O}$	11.34
Tincalconite	$\text{Na}_2\text{B}_4\text{O}_7\cdot 5\text{H}_2\text{O}$	15.16
Ulexite	$\text{NaCaB}_5\text{O}_9\cdot 8\text{H}_2\text{O}$	13.34

(Parks & Edwards, 2005)

When the acid dissociation constant of boric acid (5.81×10^{-10} at 25 °C; $\text{pK}_a=9.24$) is considered, it can be predicted that H_3BO_3 is the predominant form at neutral and low pHs whereas $\text{B}(\text{OH})_4^-$ is expected to be present at high pHs. Both forms may exist in equilibrium (1.1) at a pH range of 7.0-11.5 (Darbouret & Kano, 2000).



1.2.1 Precautions of Boron

Boron is an important micronutrient for plants, animals and humans. But the range between deficiency and toxicity is very narrow and it can be toxic at high concentrations (Kaftan, Acikel, Eroglu, Shahwan, Artok, Ni, 2005). Boron concentration recommended for drinking water is 0.5 mg/L (WHO, 2003) and is 0.75 mg/L for irrigation water (Rowe & Abdel-Magid, 1995).

Borax is necessary in small amounts for plant growth, one of the 16 essential nutrients. Borates can be used as non-toxic and non-specific herbicides. Borates are non-toxic to animals. Borates are more toxic to insects than to mammals. It is not an element that is intrinsically poisonous, but toxicity depends on structure. Today it has been scientifically demonstrated that boron is important to brain function, especially in enhancing memory, cognitive function, and hand-eye coordination (Penland, 1998).

Humans can be exposed to boron through fruit and vegetables, water, air and consumer products such as cosmetics and laundry products. When humans consume large amounts of boron-containing food, the boron concentrations in their bodies may rise to levels that can cause health problems. Boron can infect the stomach, liver, kidneys and brains and can eventually lead to death. When exposure to small amounts of boron takes place irritation of the nose, throat or eyes may occur (Draggan, 2008). Boron can also represent reproductive hazards and has suspected teratogenetic effects on humans (Bektaş, Oncel, Akbulut & Dimoglo, 2004).

1.2.2 Applications of Boron

Boron and its compounds are used for many different purposes in industry (Bektaş et al., 2004, Parks & Edwards, 2005, Jiang et al., 2006, Staroszczyk, 2009).

- To make glass, ceramics, and enamels, including fiberglass for insulation.
- As a chemical to make boron nitride, one of the hardest known substances, for abrasives and cutting tools.
- In borosilicate glasses. "Pyrex" is a common trade name for a borosilicate glass. This glass is chemically resistant, and has a small coefficient of thermal expansion.
- In porcelain enamels for iron, and for tiles and sanitary ware.
- Boron gives a blue-green flame, and the brown amorphous form is often used in pyrotechnical devices for this purpose.
- In agricultural chemicals, pest controls, fire retardants, fireworks and various minor applications.
- Boron compounds are used to make water softeners, soaps and detergents.
- Boron carbide is an excellent abrasive.
- Boric acid solution is used as an antiseptic, especially as eyewash.
- Boric acid is also traditionally used as an insecticide, notably against ants or cockroaches.
- Boron compounds are being used as components in sugar-permeable membranes, carbohydrate sensors and bio conjugates.
- Medicinal applications being investigated include boron neutron capture therapy and drug delivery.
- In the nuclear industry as a moderator for neutrons.
- Hydrides of boron are oxidized easily and liberate a considerable amount of energy. They have therefore been studied for use as possible rocket fuels.
- Boron is used in cosmetics, food preservatives, photographic chemicals, fireproofing fabrics, weather proofing for woods and leather production.

1.2.3 Boron Complexes

Boronic acids are known to form complexes with diols and polyhydroxy compounds, rapidly and reversibly in basic aqueous media (Kaftan et al., 2005). The interaction between polyhydroxy compounds and boric acid has been of interest (Tyman & Mehet, 2003).

Yin, Li, J., Liu & Li, Z. (2005) used boric acid as a cross-linking agent in order to obtain starch cross-linked with PVA. The hydroxyl groups in boric acid and starch/PVA react to form an ester linkage.

More recent experimental studies have been concentrated on the complexation and extraction of boric acid by monohydric alcohols, such as 2-ethylhexanol, isoamyl alcohol and notably by lipidic diols, 2-ethylhexane-1,3-diol (Poslu & Dudeney, 1983), nonane-1,3-diol, decane- and dodecane-1,3-diols, and in the aromatic *o*-hydroxymethylphenolic series with 2-chloro-6-hydroxymethyl-4-isoctylphenol (Tyman & Mehet, 2003).

Saccharides, having prearranged cis-diols, form stronger complexes with boronic acids (Sandanayakea, Jamesa & Shinkaia, 1996). Matsumoto, Matsui & Kondo (1999) developed a chitosan resin, modified by saccharides, where chitosan derivatives incorporating saccharides were synthesized by reductive N-alkylation, and the products were cross-linked with ethylene glycol diglycidyl ether and investigate the adsorption mechanism of boron on this resin.

Inukai, Kaida & Yasuda (1997) investigated the adsorption behavior of germanium (IV), tellurium (VI) and boron on branched-saccharide-chitosan resins and beads.

Bıcak and Senkal reported a sorbitol containing polymer resin (cross-linked polystyrene-divinylbenzene as base material and glycidyl methacrylate based cross-linked polymers containing *N*-methyl-d-glucamine for boron removal (Bıcak & Senkal, 1998, Bıcak, Ozbelge, Yilmaz & Senkal, 2000, Bıcak, Bulutcu, Senkal & Gazi, 2001, Sabarudin et al., 2005).

Kaftan et. al. (2005) synthesized a novel sorbent for boron removal, glucamine-modified MCM-41, an inorganic support material. Maeda, Egawa & Jyo (1995) reported boric acid adsorbent, which was prepared by the addition of tris (hydroxymethyl) aminomethane to epoxy groups in macroreticular glycidyl methacrylate-divinylbenzene copolymer beads. Sabarudin et. al. (2005) synthesized a chitosan resin derivatized with *N*-methyl-d-glucamine (CCTS-NMDG) by using a cross-linked chitosan (CCTS) as base material for adsorption/concentration of boron.

1.3 Hydrogels

Hydrogels are hydrophilic natured three-dimensional networks held together by chemical or physical bonds and capable of absorbing large amounts of water. These polymeric materials do not dissolve in water at physiological temperature and pH but swell considerably in an aqueous medium (Pal, Banthia & Majumdar, 2007). Water absorbed by hydrogel is not released under ordinary pressure. Hydrophilic groups such as hydroxyl (OH) and carboxyl (COOH) on the polymer chains absorb and store water. If enough interstitial space exists within the network, water molecules can become trapped and immobilized, filling the available free volume (Pal, Banthia & Majumdar, 2006a).

Hydrogels may be chemically stable or they may degrade and eventually disintegrate and dis-solve. They are called 'reversible', or 'physical' gels when the networks are held together by molecular entanglements, and/or secondary forces including ionic, H-bonding or hydrophobic forces. All of these interactions are reversible, and can be disrupted by changes in physical conditions such as ionic strength, pH, temperature, application of stress, or addition of specific solutes that compete with the polymeric ligand for the affinity site on the protein. Physical hydrogels are not homogeneous, since clusters of molecular entanglements, or hydrophobically- or ionically-associated domains, can create inhomogeneities (Hoffman, 2002).

Hydrogels are called 'permanent' or 'chemical' gels when they are covalently-cross-linked networks. Chemical hydrogels may also be generated by cross-linking of water-soluble polymers, or by conversion of hydrophobic polymers to hydrophilic polymers plus cross-linking to form a network. Sometimes in the latter case cross-linking is not necessary. Like physical hydrogels, chemical hydrogels are not homogeneous (Figure 1.5). They usually contain regions of low water swelling and high crosslink density, called 'clusters', that are dispersed within regions of high swelling, and low crosslink density. This may be due to hydrophobic aggregation of cross-linking agents, leading to high crosslink density clusters.

In some cases, depending on the solvent composition, temperature and solids concentration during gel formation, phase separation can occur, and water-filled ‘voids’ or ‘macro pores’ can form. In chemical gels, free chain ends represent gel network ‘defects’ which do not contribute to the elasticity of the network. Other network defects are chain ‘loops’ and entanglements, which also do not contribute to the permanent network elasticity (Hoffman, 2002).

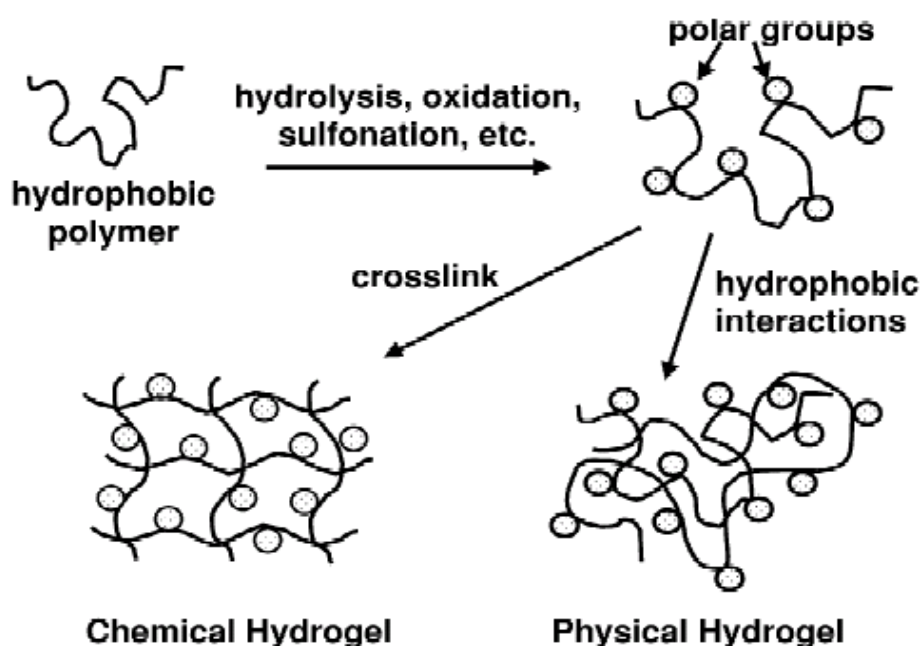


Figure 1.5 Schematic of methods for formation of hydrogels by chemical modification of hydrophobic polymers. In either case the resulting gel may be subsequently covalently cross-linked

The increasing importance of hydrogels in areas such as pharmaceuticals, food chemistry, medicine, and biotechnology has stimulated theoretical and experimental work on the properties of hydrogels in aqueous solutions. Applications of hydrogels in the biomedical field include contact lenses, blood contact materials, artificial skin and corneas, wound dressing, coating for sutures, catheters, superficial burns, donor areas, the treatment of chronic wounds containing light exudates and electrode sensors (Pal et al., 2007, Pal, Banthia & Majumdar, 2008, Piacquadio, 1994)

Hydrogels have long been used as biomaterials due to their permeability of small molecules, soft consistency, low interfacial tension, facility for purification and mainly high equilibrium water content, which make them similar in terms of physical properties to living tissues (Pal, Banthia & Majumdar, 2006b).

Hydrogels of natural polymers, especially polysaccharides such as starch, have been used recently because of their unique advantages. Polysaccharides are, in general, non-toxic, biocompatible, biodegradable and abundant. The main disadvantage of polysaccharides is their easy solubility in water which limits its ability to form stable hydrogel. One effective method to avoid these limitations is to combine them into a synthesized polymer blend hydrogels, which is becoming a subject of academic as well as of industrial interest (Pal et al., 2006a). Because pure starch does not form a film due to its hydrophilic nature, starches could be blended with various plastic materials up to a certain amount and sheets or films could be made by conventional process. Blending has become an economical and versatile route to obtain polymers with a wide range of desirable properties (Garg & Jana, 2007).

The incorporation of PVA, a biodegradable and water-soluble crystalline polymer, into starch changes the thermo mechanical properties of the material and thus modifies the polymer structure at both the molecular and morphological levels; it is widely used because of its flexibility and good film forming capability (Sreedhar, Sairam, Chattopadhyay, Rathnam & Mohan Rao, 2005). PVA might provide a stable support medium for starch films (Jayasekara, Harding, Bowater, Christie & Lonergan, 2004).

Biodegradable starch-based plastics such as starch/PVA have recently been investigated for their great potential marketability in agricultural foils, garbage and composting bags, food packaging, in the fast food industry, and in biomedical fields (Yoon, Chough & Park, 2007).

1.4 Poly (Vinyl Alcohol)

Poly (vinyl alcohol) which can be represented as PVAL, PVOH, PVA-OH or PVA is a versatile synthetic polymer with many industrial applications, and it may be the only synthesized polymer whose backbone is mainly composed of C-C bonds (Figure 1.6) that is absolutely biodegradable. PVA is a non-ionic synthetic polymer. PVA is the most readily biodegradable of vinyl polymers (Yoon et al., 2007). PVA is one of the most important vinyl polymers, prepared by partial or complete hydrolysis of poly (vinyl acetate) because the vinyl alcohol monomer is unstable. Degree of hydrolysis is the ratio of acetate groups replaced by the hydroxyls to the total acetate groups in the polymer. As the acetate groups are replaced by hydroxyls, sites are introduced, which can form strong hydrogen bonds between intra- and intermolecular hydroxyl groups, which causes PVA to show a high affinity to water (Dilek, Özbelge, Bıçak & Yılmaz, 2002).

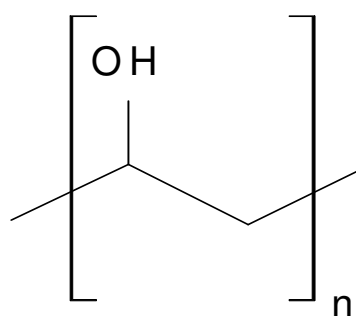


Figure 1.6 Structure of PVA

The performance properties of PVA are influenced by the molecular weight and the degree of hydrolysis. PVA has a planar zigzag structure like polyethylene. All PVA grades are readily soluble in water and solubility is dependent on factors like molecular weight, particle size distribution and particle crystallinity. As a hydrophilic polymer, PVA exhibits excellent water retention properties. Optimum solubility occurs at 87% to 89% hydrolysis (Pal et al., 2007).

PVA is of great interest due to its nontoxic, flexible, biocompatible and biodegradable properties (Wang, Chung, Lyoo & Min, 2006).

Some uses of PVA include:

- Adhesive and thickener material in latex paints, paper coatings, hair sprays, shampoos and glues.
- Children's play putty or slime when combined with borax.
- As a water-soluble film useful for packaging.
- As a surfactant for the formation of polymer encapsulated nano beads
- Used in eye drops and hard contact lens solution as a lubricant.
- Used in protective chemical-resistant gloves
- When doped with iodine, PVA can be used to polarize light.

PVA is well suited for making biodegradable blends with natural polymers. The hydrophilic nature of PVA enhances compatibility with starch, making it suitable for the preparation of polymer blends. The polarity of PVA helps the blend in accelerating the hydrolytic attack by atmospheric moisture that results in breakdown in the sugar molecules of natural polymers (Jayasekara, Harding, Bowater, Christie & Lonergan, 2003, Sreedhar et al., 2006).

In testing PVA for toxicity and for compatibility with skin and mucous membranes, no negative effects were found in animals, and no limitation for its existence in waste effluents was imposed (Dilek et al., 2002).

1.5 Objectives and Scope of the Thesis Study

Boron and its compounds are used for many different purposes in industry and are known to form complexes rapidly and reversibly in aqueous media with polyhydroxy compounds, monohydric alcohols, diols and aromatic o-hydroxymethylphenolic compounds (Tayman & Mehet, 2003).

Starch; a natural polymer, contains abundant hydroxyl groups. These hydroxyls are potentially able to react with any chemicals having reactivity with alcoholic hydroxyls. This would include a wide range of compounds such as acid anhydrides, organic chloro compounds, aldehydes, epoxy, ethylenic compounds, etc. (Ogura, 2004). The hydroxyl glucose units of starch react similarly as primary and secondary alcohols. Among others, with the involvement of these groups, starch readily esterifies inorganic and organic acids, forms ethers and metal derivatives, in which metal atoms are bound via valence bonds to the hydroxyl oxygen atoms (Tomasik & Schilling, 2004).

Chemical cross-linking is a highly versatile method to modify polymers, where properties can be improved, such as mechanical, thermal and chemical stability. Cross-linking agents enhances not only the thermal properties but also the mechanical properties of complexes by reinforcing the intermolecular binding with the introduction of covalent bonds to supplement natural intermolecular hydrogen bonds (Sreedhar et al., 2006).

Hydrogels are hydrophilic natured three-dimensional networks, capable of absorbing large amounts of water and held together by chemical or physical bonds (Pal et al., 2007). Hydrogels have long been used as biomaterials in superficial burns, donor areas and in the treatment of chronic wounds containing light exudates (Piacquadio, 1994).

Natural polymers such as starch are being recently used for the preparation of hydrogels because of their non-toxicity, biocompatibility, biodegradability and abundance in nature. However, in order to form stable hydrogels, water soluble starch needs to be mixed with a polymer capable of forming good films (Pal et al., 2006a).

The incorporation of boron in the backbone of polymers improves thermal stability, mechanical, electrical, antibacterial and antifungal properties, oxidative resistance, flexibility, flame retardancy than their virgin counterparts (Wang, Chang, & Chen, 2008, Martin, Hunt, Ebdon, Ronda & Cadiz, 2006, Gao, Su & Xia, 2005, Gao, Liu & Wang, 2001, Martín, Ronda, & Cádiz, 2006, Uslu, Daştan, Altaş, Yayli, Atakol & Aksu, 2007).

In this study we attempted to prepare boron complexes of starch which were unmodified or modified by cross-linking reactions. Complexes were obtained either in powder or hydrogel forms. PVA was used to provide a stable support medium for starch hydrogels due to its flexibility and water solubility. GA, EPI and Z-6020 were used as cross-linking agents.

The characterization of the prepared complexes were realized by Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), scanning electron microscope (SEM), nuclear magnetic resonance spectroscopy (^{11}B -NMR) and X-ray diffraction analysis (XRD) methods. Degree of solubility, swelling and TS tests were performed for synthesized complexes.

In this work we also attempted to investigate the *in vitro* antimicrobial activities of prepared boron-starch powder and hydrogel complexes. Antimicrobial activities of the complexes were assayed for five different bacterial cultures and one fungus. Standard disks of amoxicillin/clavulanic acid (AMC), ofloxacin (OFX), netilmycin (NET), erythromycin (ER) and amphotericin B (AFB) were individually used as positive controls during antimicrobial activity testes.

CHAPTER TWO

MATERIALS AND METHOD

2.1 Materials and Apparatus

The potato starch (Fluka 85643) and poly (vinyl alcohol) (Fluka 81384) of analytical reagent grade were used in this study and no further purification was made before use.

Epichlorohydrin (Aldrich E105-5), glutaraldehyde (Merck 8.20603) and N-(β -aminoethyl)- γ -aminopropyltrimethoxysilane (Dow Corning 2604184) were used as cross-linking agents (Figure 2.1). Boric acid (BA) (Merck 1.00160) was used for the preparation of boron complexes. All other chemicals used were of analytical reagent grade.

In all experiments, potato starch was dried in oven for 2 h at 105 °C before use.

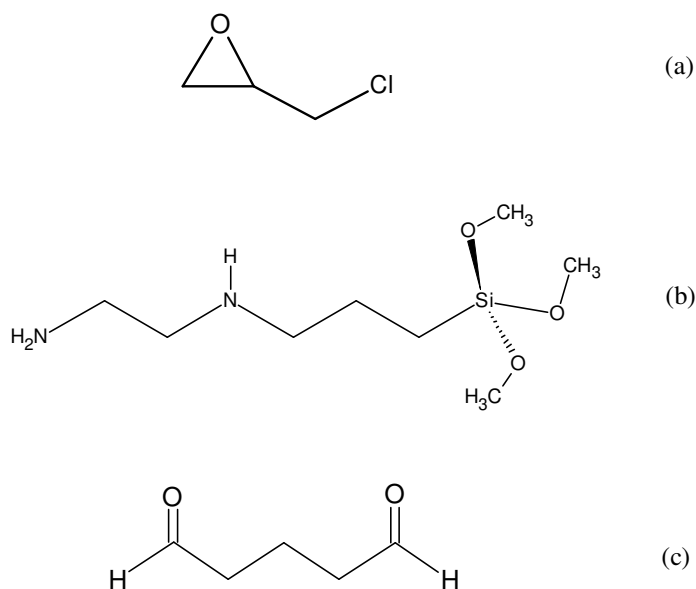


Figure 2.1 Structures of (a) EPI, (b) Z-6020 and (c) GA

GA reagent solution was prepared by mixing 0.250 mL of reagent in a solution of 5 mL ethanol (Riedel_de Haën 07102) including 0.025 mL hydrochloric acid (Riedel_de Haën 07102). Besides, Z-6020 reagent solution was prepared by adding 0.250 mL reagent to 5 mL distilled water. 0.400 mL glacial acetic acid (Riedel_de Haën 27225) was used in order to adjust the pH to 3.5.

Definite pH solutions (pH: 2.5-8) used in the investigation of swelling behaviors of cross-linked starch/PVA hydrogels were prepared by using citric acid (Merck 1.00242) and disodium hydrogen phosphate (Merck 1.06580) at constant ionic strength ($I=0.01$).

A Denver 215 model pH meter, a Heidolph MR 3001 model magnetic shaker, Retsch PM 200 model grinder and a Polyscience 9006 model refrigerating-heating circulator were used during the experiments.

2.2 Preparation of Cross-linked Starch (CLS) Powders

5 g of starch portions were dispersed in 10 mL distilled water so as to obtain a solid concentration of 50% (w/v) and the pastes were stirred mechanically. Then the cross-linkers were individually added to these pastes. When EPI was used the pH of the slurry was adjusted to 10.5 using sodium hydroxide solution (1M) and EPI was then added to give 1.0% (0.042 mL) final concentration based on the starch dry weight (Jyothi, Moorthy & Rajasekharan, 2006). The prepared GA and Z-6020 reagent solutions were directly added to the pastes in acidic medium. The flasks were stoppered well and the contents were stirred at 45 °C for 2 h. Upon completion of the reactions, the pH of the slurry was adjusted to 6 using 1 M HCl for EPI. The products were washed with distilled water by using dialysis membrane. The slurries were filtered; the products were dried overnight in an air oven at 45°C and were then ground at 350 rpm for 10 min. The powders so obtained (Figure 2.2 and 2.3) were named as CLS-EPI, CLS-GA and CLS-Z 6020 respectively.

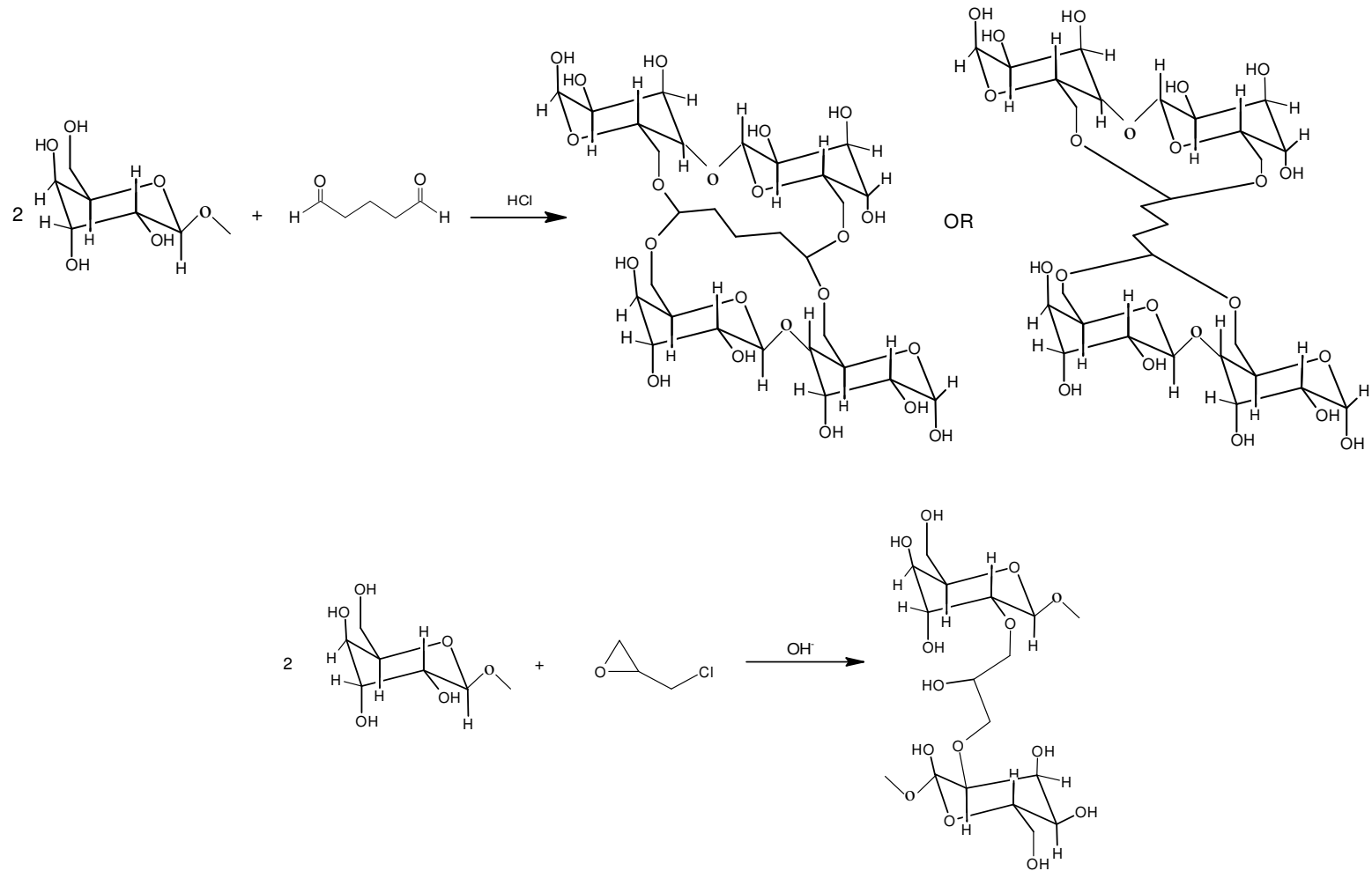


Figure 2.2 Hypothetically proposed reaction scheme for preparation of cross-linked starch powders with (a) GA and (b) EPI (Ramaraj, 2007, Singh et al., 2007)

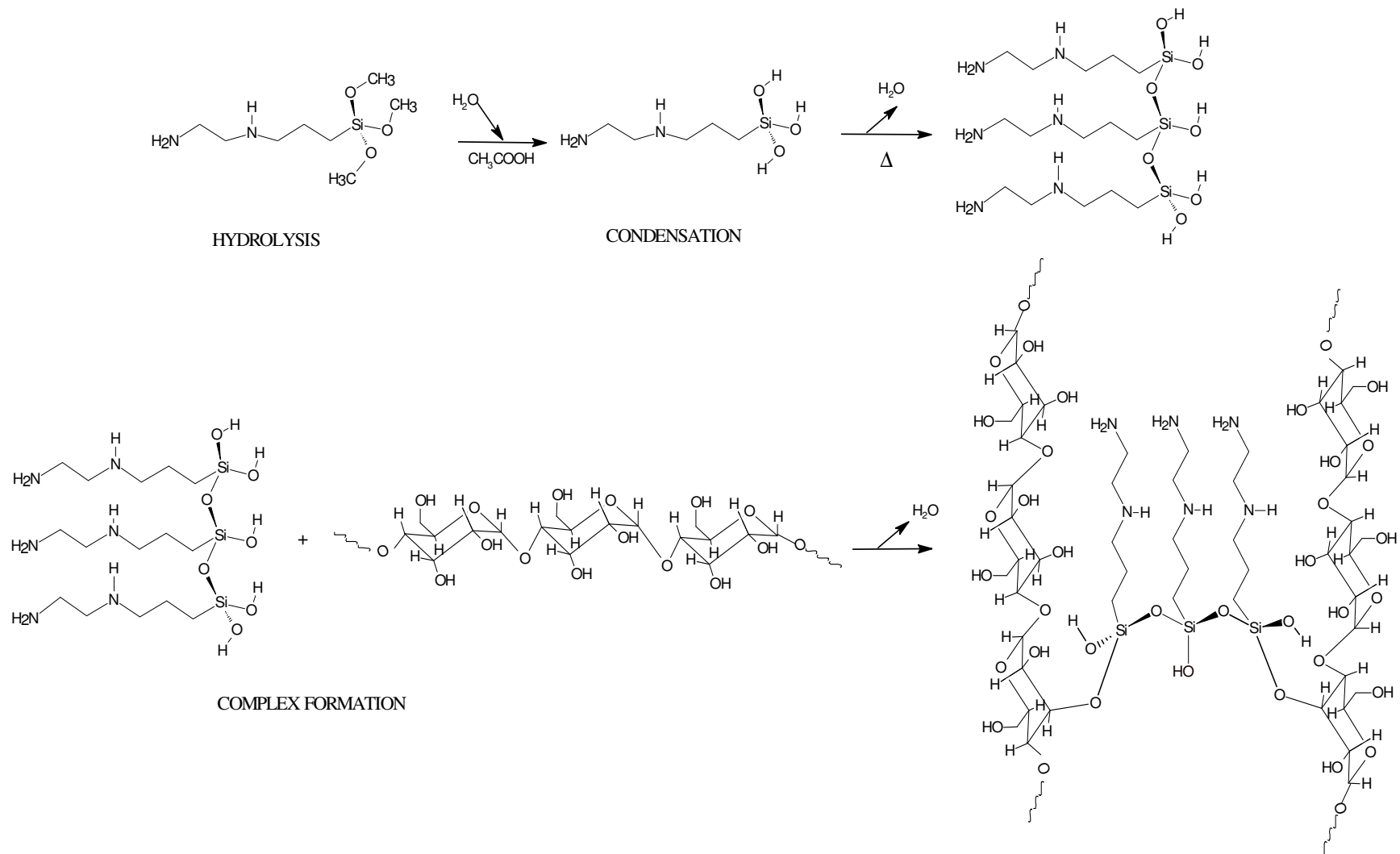


Figure 2.3 Hypothetically proposed reaction scheme for preparation of cross-linked starch powders with Z-6020

2.3 Preparation of Starch/PVA Hydrogels (SF)

During the preparation of the starch/PVA hydrogels aqueous PVA solution was blended with aqueous gelatinized starch solution. To this mixture GA and Z-6020 in acidic and EPI in alkali medium were added. Then the hydrogels were obtained by solution casting technique.

Numerous dehydration temperatures between 15-50 °C in vacuum and air oven were examined but the films could not be separated from the petri dishes when dried in these conditions. So the hydrogels were dried at room temperature (RT) in order to avoid this limitation.

25 mL 10% (w/v) aqueous PVA solutions were blended with 25 mL 5% (w/v) aqueous gelatinized starch solutions at 70 °C for 1h with constant stirring at 400 rpm to get homogeneous mixtures. To these mixtures 0.250 mL EPI, 5.275 mL GA (Pal, et.al, 2008) or 5.650 mL Z-6020 reagent solutions were individually added. When EPI was used the pH of the solution was adjusted to 10.5 with 1M NaOH. The mixtures were refluxed at 70 °C for 2 h at 1250 rpm. The prepared blends were casted into glass petri dishes and dried at RT.

The obtained transparent films (Figure 2.4) were named as SF-EPI, SF-GA and SF-Z 6020, respectively.

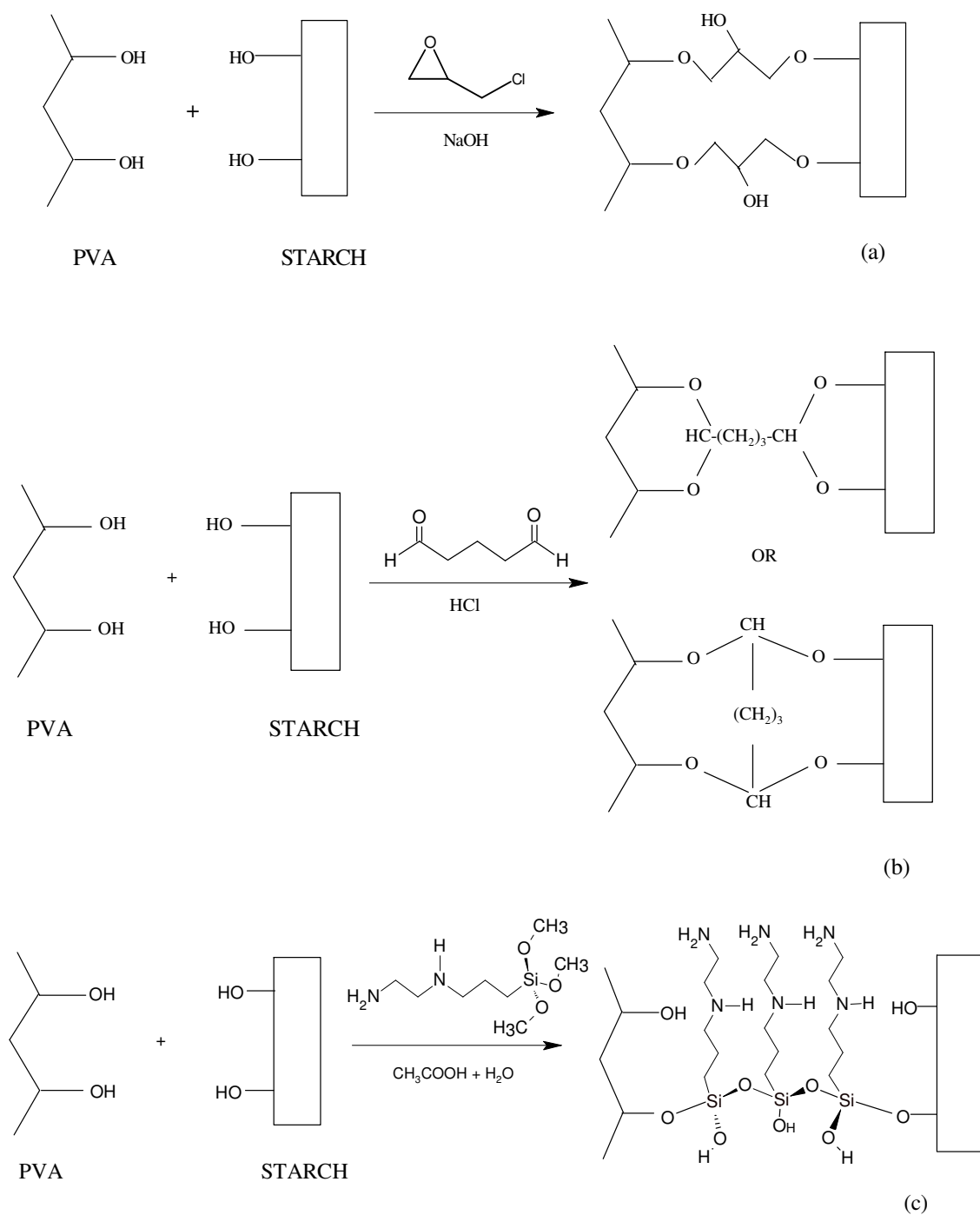


Figure 2.4 Hypothetically proposed reaction scheme for preparation of starch/PVA hydrogels cross-linked with (a) EPI, (b) GA and (c) Z-6020

2.4 Preparation of Boron-Starch Powder Complexes

The boron atom in BA can be considered as an electron-deficient atom and therefore, very reactive to any group which can donate electrons and thereby stabilize the boron atom. So, BA functions as an electron acceptor, giving the tetrahedral borate anion. Thus starch, as a polyalcohol, can react with this acid to form esters (Staroszczyk, 2009). The interaction between starch and BA or $B(OH)_4^-$ was shown in Figure 2.5.

Starch and boron complexes were prepared in two ways; with or without using cross-linking agents. 5 g starch was dispersed in 5 mL distilled water and the pH of the slurry was adjusted to 10.5 with 1M NaOH. Then 5 mL aqueous solution of BA (0.0375 g) and 0.042 mL of EPI were added to this slurry. The slurry was refluxed at 350 rpm and 45 °C for 2h. The pH of the slurry was adjusted to 6 using 1 M HCl.

Otherwise; BA (0.0375 g) was dissolved in 10 mL distilled water or mixture of 5 mL distilled water - Z-6020 or GA reagent solutions. Then 5 g starch was dispersed in these solutions and the pastes were refluxed at 350 rpm and 45 °C for 2h. All the slurries were washed with distilled water by using dialysis membrane, dried overnight in an air oven at 45 °C and then ground at 350 rpm for 10 min. The powders so obtained were named as CLS-EPI-BA, S-BA, CLS-GA-BA and CLS-Z 6020-BA, respectively.

2.5 Preparation of Boron and Starch/PVA Hydrogels

Boron complexes of starch/PVA hydrogels were prepared in two ways; with or without using cross-linking agents.

25 mL 10% (w/v) aqueous PVA solutions were blended with 25 mL 5% (w/v) aqueous gelatinized starch solutions at 70 °C for 1h with constant stirring at 400 rpm to get homogeneous mixtures.

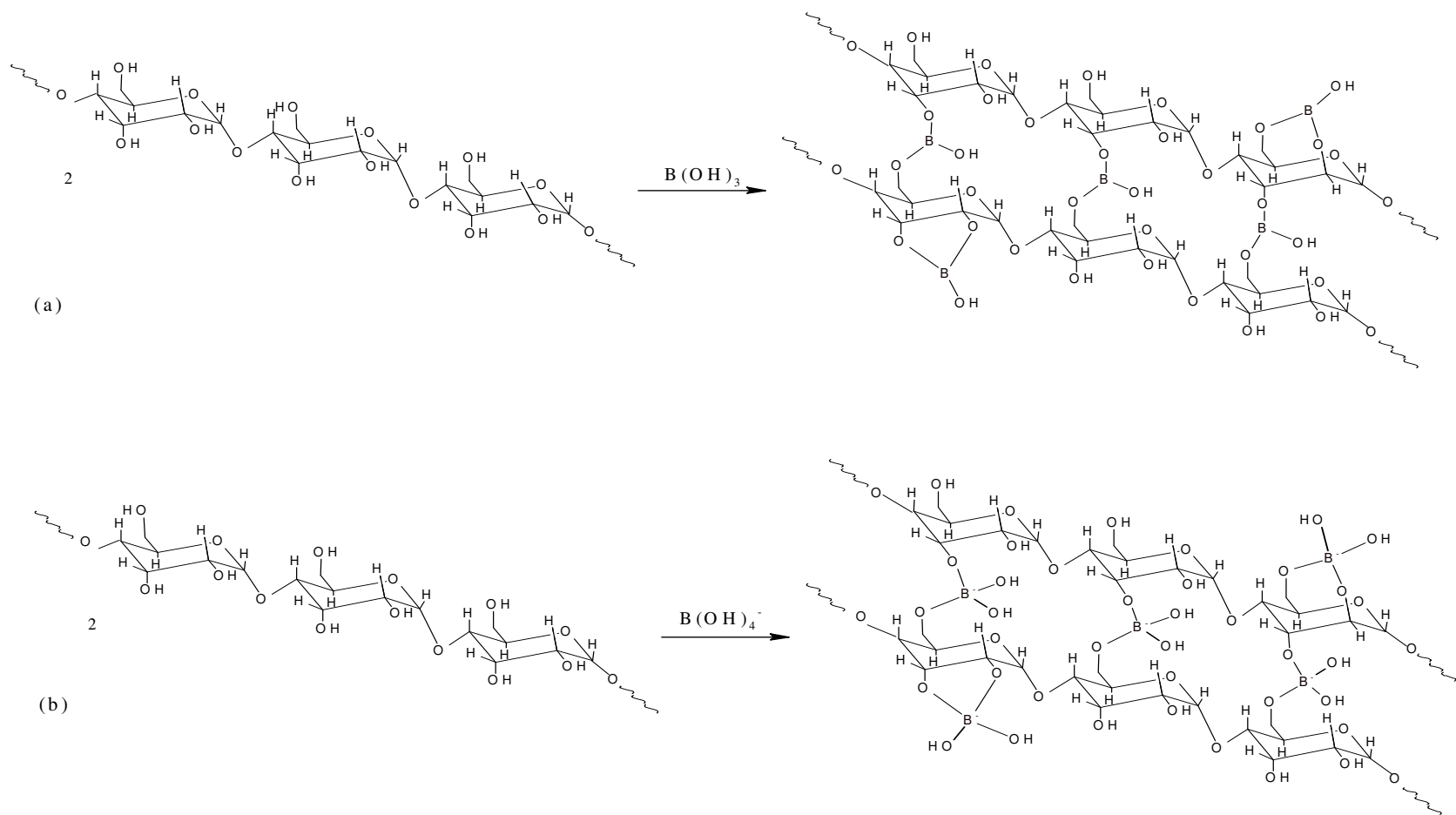


Figure 2.5 Hypothetically proposed reaction scheme for complexation between boron species and starch (a) $B(OH)_3$ and (b) $B(OH)_4^-$ (Kaftan et al., 2005)

To these mixtures; 0.0375 g BA or 0.250 mL EPI-0.0375 g BA or GA reagent solution-0.0375 g BA or Z-6020 reagent solution-0.0375 g BA blends were individually added. When EPI was used the pH of the slurry was adjusted to 10.5 with 1M NaOH. The flasks were stoppered well and the contents were stirred and refluxed at the same temperature for 2 h at 1250 rpm. The solutions were casted into glass petri dishes and dried at RT.

The films so obtained were transparent and named as SF-BA, SF-EPI-BA, SF-GA-BA and SF-Z-6020-BA, respectively.

2.6 pH Dependent Swelling Behaviors of Synthesized Hydrogels

In order to investigate the swelling behavior of the hydrogels, the swelling equilibrium times at ultra pure water and citric acid-phosphate solutions of definite pH values (2.5; 4.5; 6.5 and 8) were studied initially. The dried samples were immersed in the solutions at RT and removed at intervals of 5 min, dried with filter paper to remove surface water, weighed and then returned to the same solutions until the equilibrium was reached.

The pH dependent swelling behavior of the membranes were determined by swelling up the dried membranes in water until equilibrium times and then immersing the same products in the citric acid-phosphate solutions of different pH values, respectively and standing up the membranes for maximum determined equilibrium time in each solution.

The swelling ratios were calculated on a dry basis using the equation (2.1); where W_h is the weight of the product after hydration and W_d is the weight of the dried product. The experiments were conducted in triplicates and the results were given as averages.

$$\text{Swelling ratio} = [(W_h - W_d) / W_d] \cdot 100 \quad (2.1)$$

2.7 Solubility Tests for Synthesized Powder Complexes

Solubility tests were carried out to confirm the completion of the cross-linking in starch and to investigate the effect of the amount of cross-linker used and effect of complexation with BA. For this purpose cross-linked potato starches were also prepared by increasing the amount of cross-linking reagents two times in the same manner as described above in part 2.2. The powders so obtained were named as CLS-EPI(2), CLS-Z 6020(2) and CLS-GA(2).

All synthesized powder complexes were weighed to the nearest 0.1 g and placed into beakers with 10 mL ultra pure water. The samples were maintained under constant agitation at 450 rpm for 3 h at RT. Then the samples were collected by filtration and dried in an air oven at 50 °C to constant weight.

The percentage of total soluble matter (%solubility) was calculated using the equation 2.2; where W_i is the initial weight of the dry product and W_d is the final weight of the dry product.

$$\% \text{ Solubility} = [(W_i - W_d)/W_i] \cdot 100 \quad (2.2)$$

2.8 In vitro Antimicrobial Activities of Synthesized Complexes

2.8.1 Test Microorganisms

The *in vitro* antimicrobial activities of synthesized complexes were tested against laboratory control strains belonging to the American Type Culture Collection (Maryland, USA): *Escherichia coli* [ATCC 25922], *Staphylococcus aureus* [ATCC 25923], *Streptococcus pyogenes* [ATCC 19615], *Pseudomonas aeruginosa* [ATCC 27853], *Bacillus subtilis* [ATCC 11774], and one fungus, *Candida albicans* [ATCC 10231].

2.8.2 Evaluation of Antimicrobial Activity

Antimicrobial activity tests were assayed by the disc diffusion susceptibility test according to the recommendation of the National Committee for Clinical Laboratory Standards (NCCLS) (Clark, Jacobs & Appelbaum, 1998). The disk diffusion tests were performed on Muller-Hinton agar plates. Plates were dried at 35 to 36 °C for about 30 min in an incubator before inoculation. Three to five freshly grown colonies of bacterial strains were inoculated into 25 mL of Muller-Hinton broth medium in a shaking water bath for 4 to 6 h until a turbidity of 0.5 McFarland (1×10^8 CFU/mL) was reached. Final inocula were adjusted to 5×10^5 CFU/mL. Three to five colonies of *C. albicans* were inoculated into 25 mL of Sabouraud dextrose broth in a shaking water bath for 8 to 10 h until a turbidity of 0.5 McFarland was reached. The final inocula were adjusted to 5×10^5 CFU/mL using a spectrophotometer (Kirkpatrick, Turner, Fothergill, McCarthy, Redding, Rinaldi & Patterson, 1998). The inoculum (50 µL) from the final inocula was applied to each agar plate and uniformly spread with a sterilized cotton spreader over the surface. Absorption of excess moisture was allowed to occur for 30 min before application of hydrogel and powder samples.

Hydrogel discs (9 mm) were directly applied onto agar plates. For powder samples which were not soluble in DMSO, holes were opened with a diameter of 9 mm on the agar plates under sterile conditions and portions of 5 mg powder samples were placed into these holes. For the other soluble powder samples; sterile filter-paper disks (Oxoid, England, 6 mm in diameter) were impregnated with 20 µL of the sample solutions in dimethylsulphoxide (DMSO), 5 mg per 1 mL of DMSO (all solutions were filter sterilized using a 0.20 µm membrane filter) and placed on inoculated plates. These plates were incubated at 37 °C for 24 h for bacteria and 48 h for fungi.

Standard disks of amoxicillin/clavulanic acid (AMC, 30 μ g/disc), ofloxacin (OFX, 5 μ g/disc), netilmycin (NET, 30 μ g/disc), erythromycin (ER, 15 μ g/disc) and amphotericin B (AFB, 30 μ g/dics) were individually used as positive controls and the disks imbued with 20 μ L of pure DMSO were used as a negative control. The diameters of the inhibition zones were measured in millimeters using an inhibition zone ruler.

2.9 Characterization Techniques of Complexes

2.9.1 XRD Analysis

The X-ray diffraction patterns (XRD) of the complexes were recorded with oriented mounts, in a Philips X'Pert Pro X-Ray diffractometer using Cu K α radiation at 45 kV and 40 mA in the 2θ range of 0-60 $^\circ$.

2.9.2 FTIR Analysis

The FTIR analyses of the samples were conducted with Perkin–Elmer Spectrum BX-II Model FTIR spectrophotometer. All samples were dried to a constant weight in an air oven at 50 $^\circ$ C for 24 h before use and KBr pellets were recorded in the range of 4000 and 400 cm^{-1} , at a resolution of 4 cm^{-1} as an average of 50 scans.

2.9.3 Thermal Analysis

To have views on thermal stability of starch, PVA, synthesized cross-linked starch powders and the hydrogels, TGA analysis of the samples were carried out with Perkin Elmer Diamond TG/DTA Analyzer. The analyses were made in aluminum pans under a dynamic nitrogen atmosphere in temperature range of 25-600 $^\circ$ C at a heating rate of 10 $^\circ$ C/min.

2.9.4 SEM Analysis

The surfaces of the complexes were observed with an emission scanning electron microscope. The samples were coated with a thin gold layer (two times, 40 mA, 60 s; approx. 30 nm) by a sputter coater unit (BALZER SCD 050 Sputter Coater, BAL-TEC) and surface topography was analyzed with a JEOL JSM 6300F Scanning Electron Microscope (SEM) operated at an acceleration voltage of 5 kV for powder complexes and 10 kV for hydrogels.

2.9.5 ^{11}B -NMR Analysis

The ^{11}B -Nuclear Magnetic Resonance spectra of the boron complexes in the powder form were obtained using a Bruker Avance DPX 400 model 128.18 MHz spectrometer with DMSO- d_6 as solvent.

The ^{11}B -NMR spectra showed a broad signal at chemical shifts between 50 and -50 ppm for all compounds and proper signals couldn't be achieved in these spectra because of the low solubility of the cross-linked starch-boron complexes. Depending on solubility problems, NMR spectra of the hydrogel boron complexes were not clearly observed and recorded. For this reason, the spectra of ^{11}B -NMR analysis were not shown in results part of this thesis study.

2.9.6 Mechanical Tests

The tensile strength (TS), elongation at break ($\epsilon\%$) and Young's modulus (E) of the hydrogels were determined using a AG-IS 100 kN model Shimadzu Universal tensile testing device equipped with a 5 kN load cell at a cross head speed of 0.5 mm/min. Each result was taken from 3 (replicates, $n = 3$) "dog bone" shaped specimens, (ASTM Standard Method D638-02a, 2002) and the results were given as averages. The thicknesses of the films produced were between 0.10-0.40 mm depending on the blend composition.

CHAPTER THREE

RESULTS

3.1 Characterization of starch-boron complexes

3.1.1 XRD analyses

The X-ray diffraction patterns and summary of XRD results of synthesized hydrogels and powder complexes were shown in Figures 3.1-3.10 and Tables 3.1- 3.2.

3.1.1.1 XRD Analyses of Synthesized Hydrogels

In the diffractogram of starch, only one strong diffraction peak was observed at approximately $2\theta=17.0^\circ$ which resembles to the characteristic of B-type crystalline structure (Cairns, Sun, Morris & Ring, 1995). Starch is known to be semi-crystalline in nature due to the amylopectin fraction that exists in it and have typical crystalline peaks at 16.6° and 22.0° because of its close molecular packing and regular crystallization.

PVA had peaks at $2\theta=18.9^\circ$ and 38.9° . The peak at 18.9° was most intense. The X-ray diffractograms of hydrogels revealed amorphous compounds, with intense peaks at approximately $2\theta=20.0^\circ$ indicating that the crystallinity of the membranes were mainly contributed by PVA.

The XRD analysis results showed that the morphology of starch was changed when starch was blended with PVA in the synthesized hydrogels. There appeared an increase in overall intensities of the XRD reflections with cross-linking and at the same time the crystalline peak of PVA at $2\theta=38.9^\circ$ completely disappeared.

The difference in crystallinity between powder and hydrogel samples (Figure 3.5 and 3.10 or Figure 3.4 and 3.9) is the effect of gelatinization of starch during synthesis of the hydrogels. In this case, a crystalline structure reappears, which could be also associated with the B-modification. Then the morphology of native granules is unstructured, and the final crystallinity is higher for hydrogels (Cyras et al., 2006).

When crystallinity of hydrogels which were just cross-linked with cross-linking agents and boron complexes of these hydrogels were compared (Figure 3.4 and 3.5), considering the increase in the intensities of crystalline peaks it was observed that the crystallinity and gelatinization was much for SF-GA-BA than SF-GA. But the intensities of the peaks were reduced for hydrogels SF-EPI-BA and SF-Z 6020-BA. So it could be claimed that BA complexes of the starch/PVA hydrogels crosslinked with EPI and Z-6020 were less crystalline indicating the lower gelatinization of these complexes compared to SF-EPI and SF-Z 6020.

BA peaks (as it gave single crystal peaks) could not be identified in boron containing hydrogels suggesting that few or no BA remained in a crystal state inside the microparticles of the hydrogels (Li, B., Wang, Li, D., Chiu, Zhang, Shi, Chen & Mao, 2009).

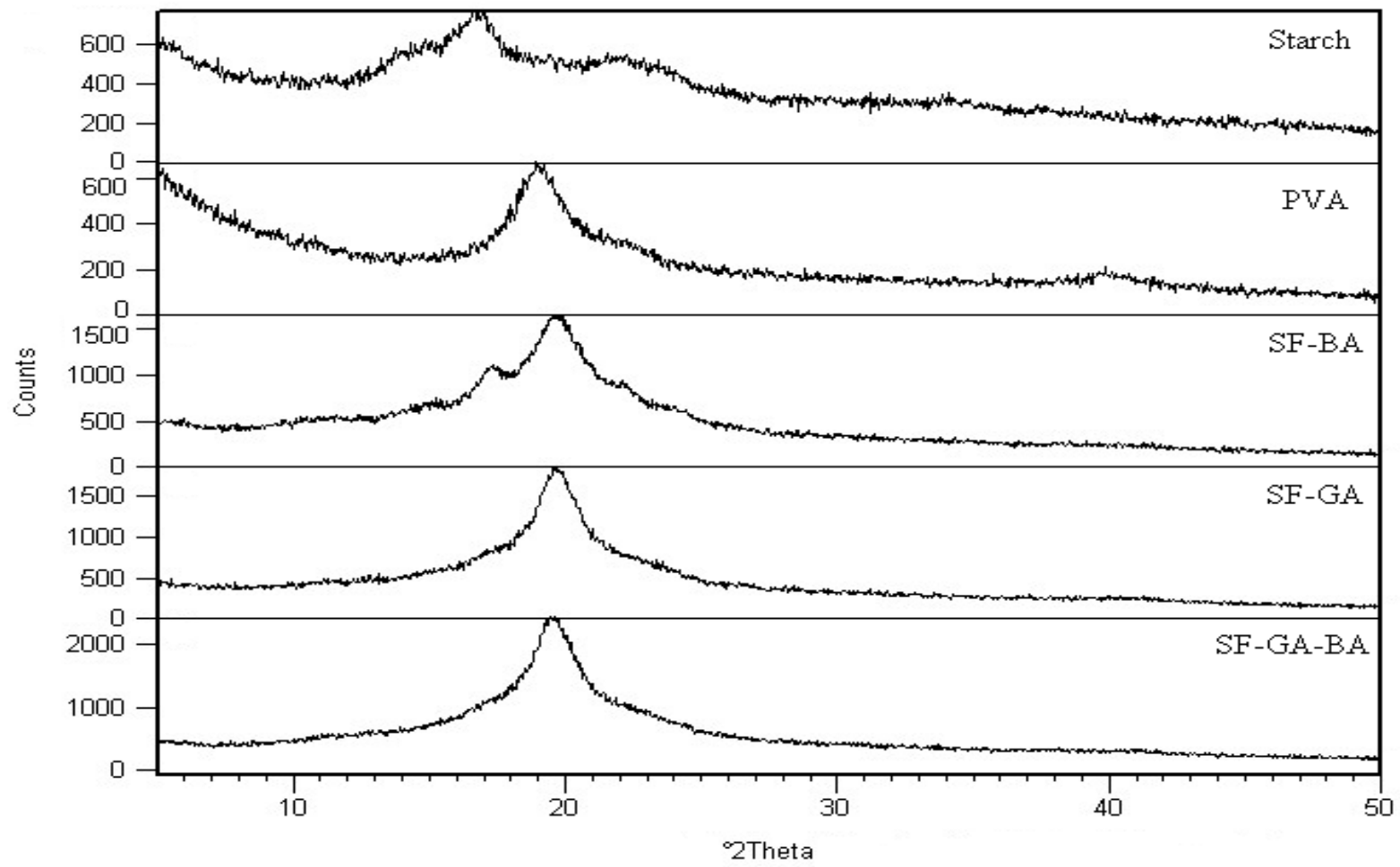


Figure 3.1 The XRD patterns of Starch, PVA, SF-BA, SF-GA and SF-GA-BA

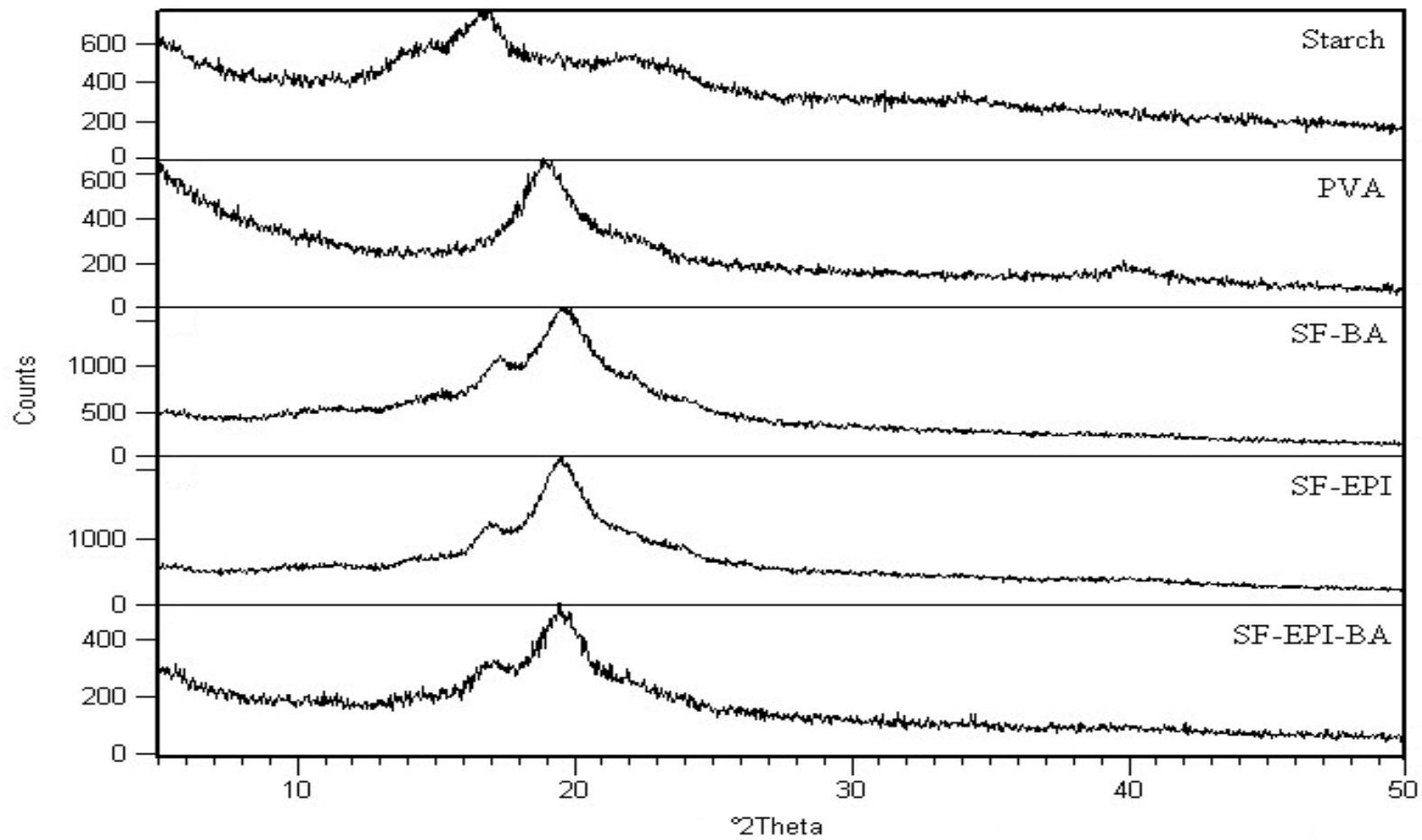


Figure 3.2 The XRD patterns of Starch, PVA, SF-BA, SF-EPI and SF-EPI-BA

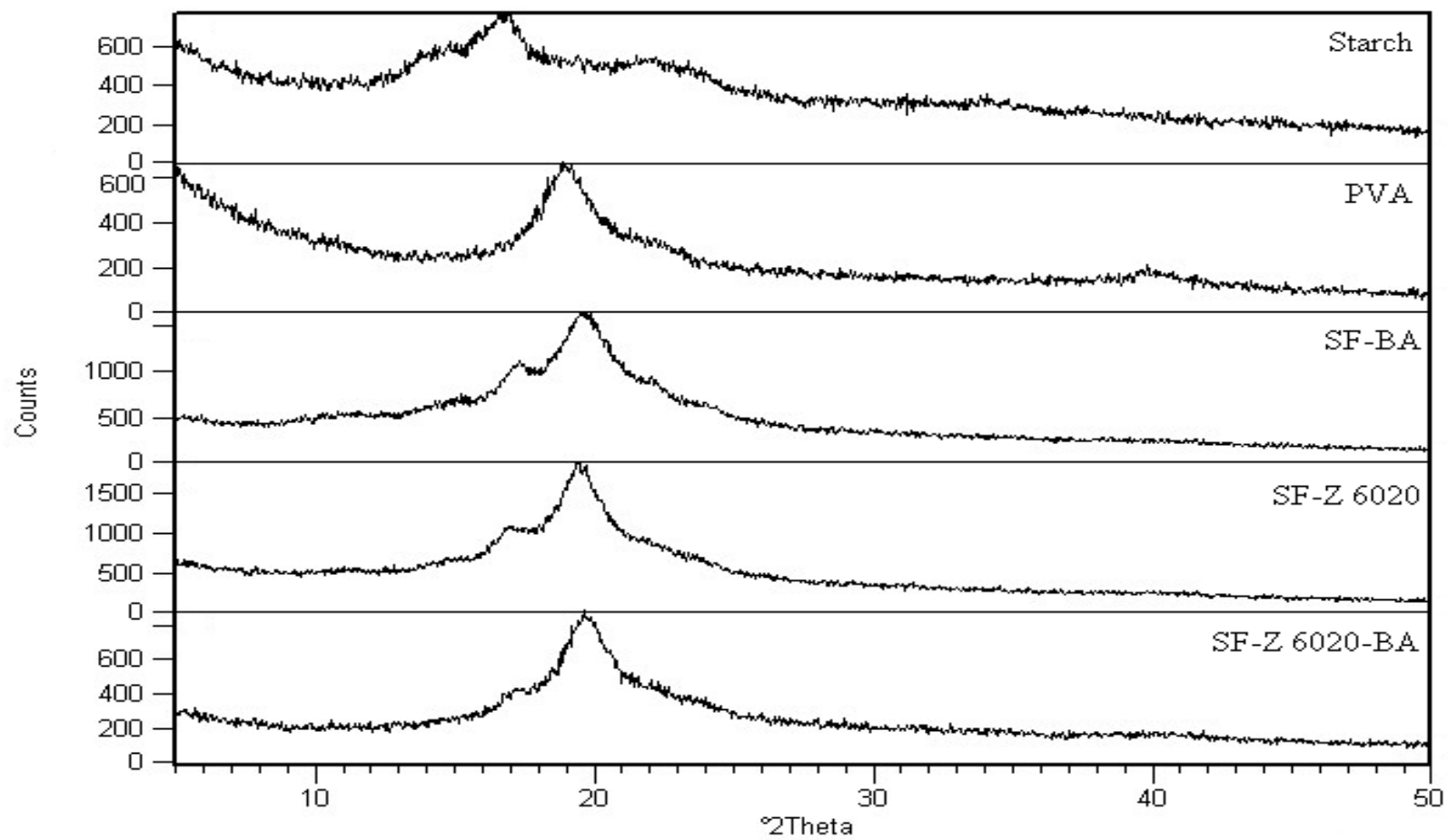


Figure 3.3 The XRD patterns of Starch, PVA, SF-BA, SF-Z 6020 and SF-Z 6020-BA

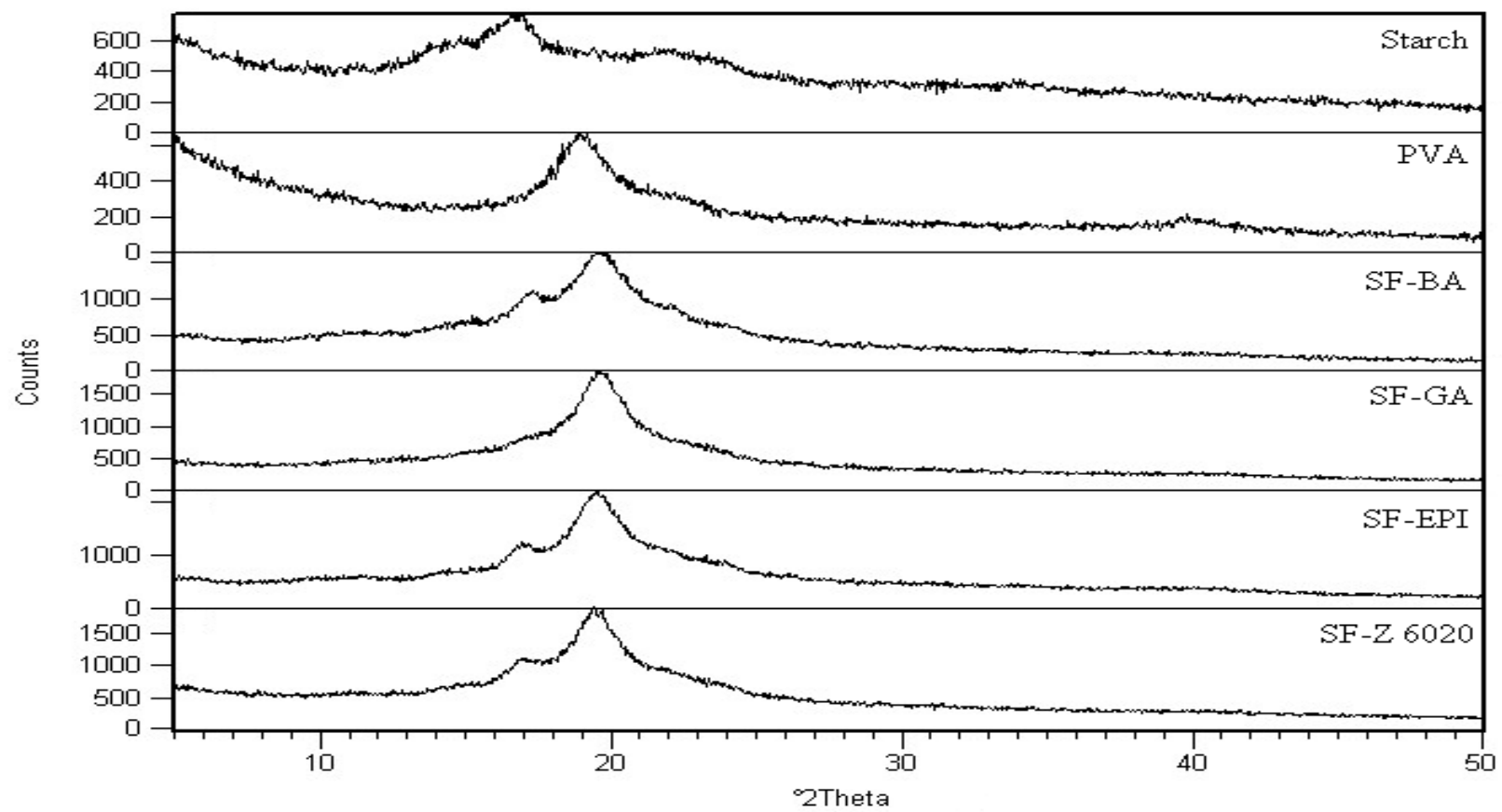


Figure 3.4 Comparison between the XRD patterns of Starch, PVA and all cross-linked hydrogels

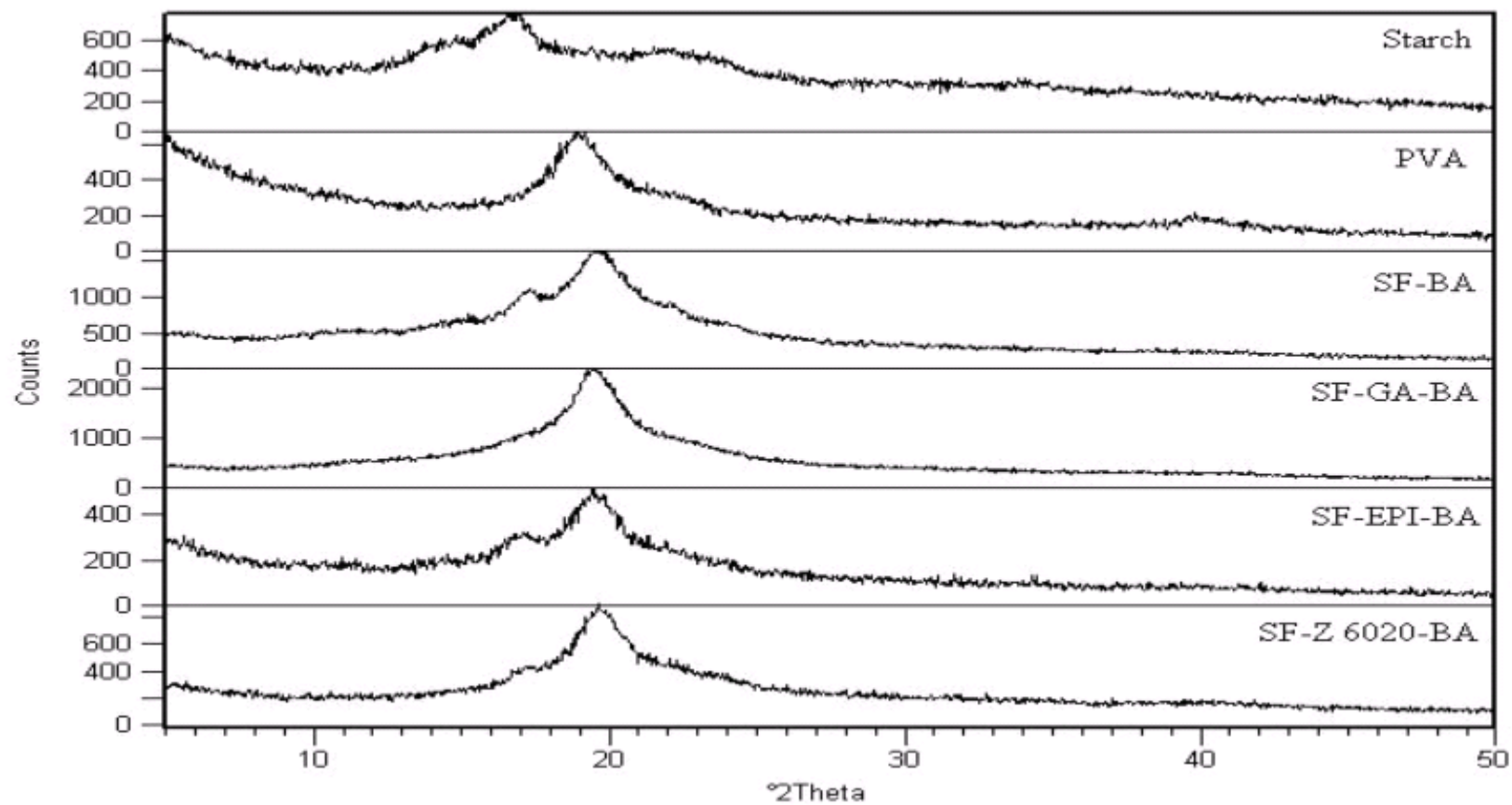


Figure 3.5 Comparison between the XRD patterns of Starch, PVA and boron complexes of cross-linked hydrogels

3.1.1.2 XRD Analyses of Synthesized Powder Complexes

The starch granule is heterogeneous both chemically (e.g., amylose and amylopectin) and physically (e.g., crystalline and non-crystalline regions). The presence or absence of crystalline order is often a basic factor underlying starch properties.

Modification of X-ray diffraction patterns for synthesized powder complexes and hydrogels could be correlated with physical and chemical transformations that occurred during gelatinization and cross-linking (Ispas-Szabo, Ravenelle, Hassan, Preda & Mateescu, 2000).

When the differences between the XRD patterns of starch and boron complexes of cross-linked starches were investigated (Figure 3.10), with increasing degree of cross-linking, the intensity of sharp peaks of diffraction occurred in the diffractogram of starch were diminished for all boron containing powder complexes. Meanwhile, their amorphous area got correspondingly wider, except CLS-Z6020-BA. The variations were attributed to the crosslinking reactions, which restricted the activity of the starch molecules, destroyed the regularity of the starch molecule, and weakened the intermolecular forces of the starch molecular chains and the hydrogen bond. Therefore, the crystallization capacity was reduced (Zhao, Li, Wang & Lai, 2008). In this case, cross-linking is claimed to be more effective in boron complexes of starches which were cross-linked with GA and EPI than Z-6020.

Additionally, crystallinities of starch and the crosslinked potato starches (Figure 3.9) were not significantly different. This means that the crystal area of the starch was not greatly affected when crosslinking agents were used alone in the synthesis without using BA. But when BA was used with different cross-linkers (Figure 3.10) a reduction in crystallinity was observed indicating the increase in cross-linking degree.

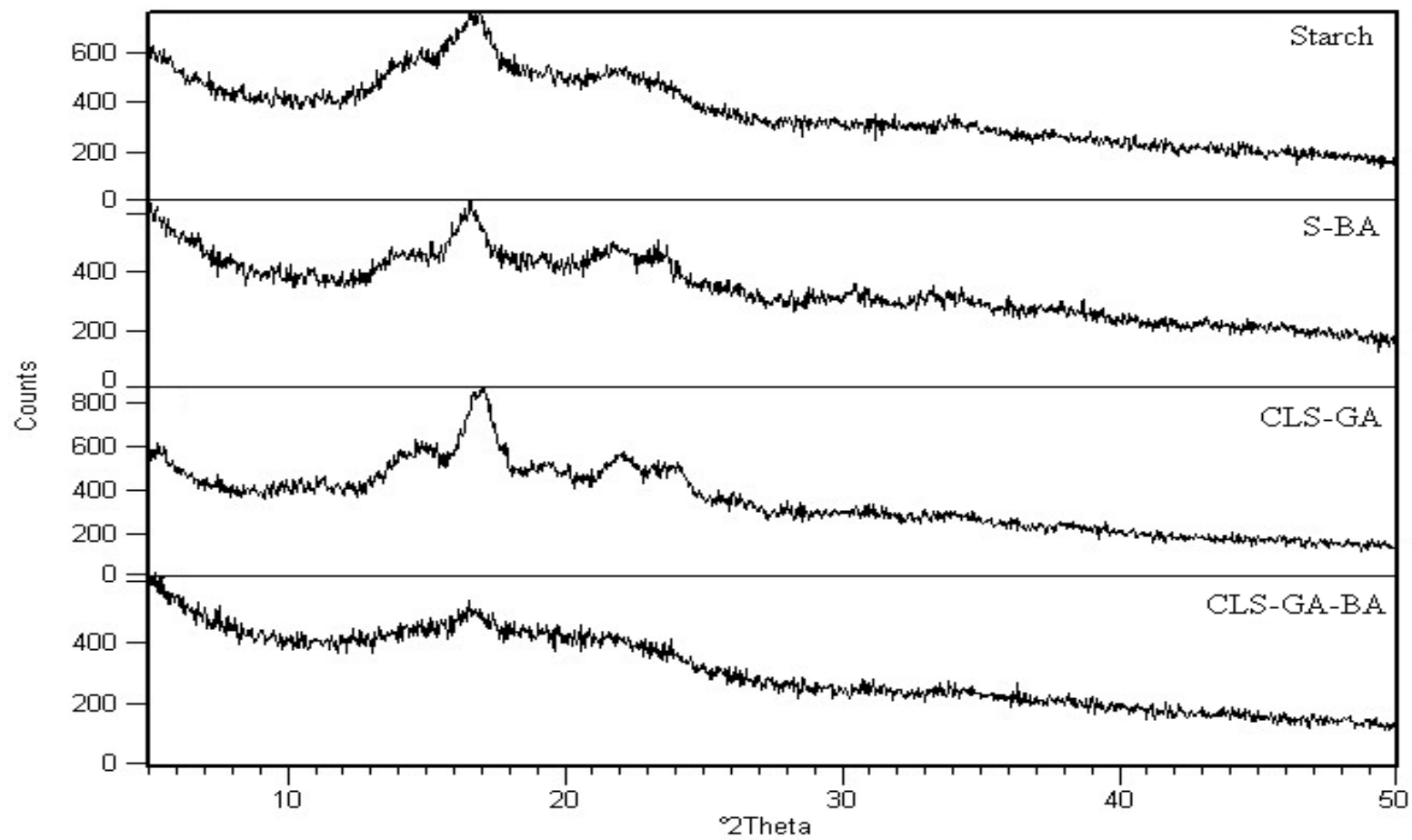


Figure 3.6 The XRD patterns of Starch, S-BA, CLS-GA and CLS-GA-BA

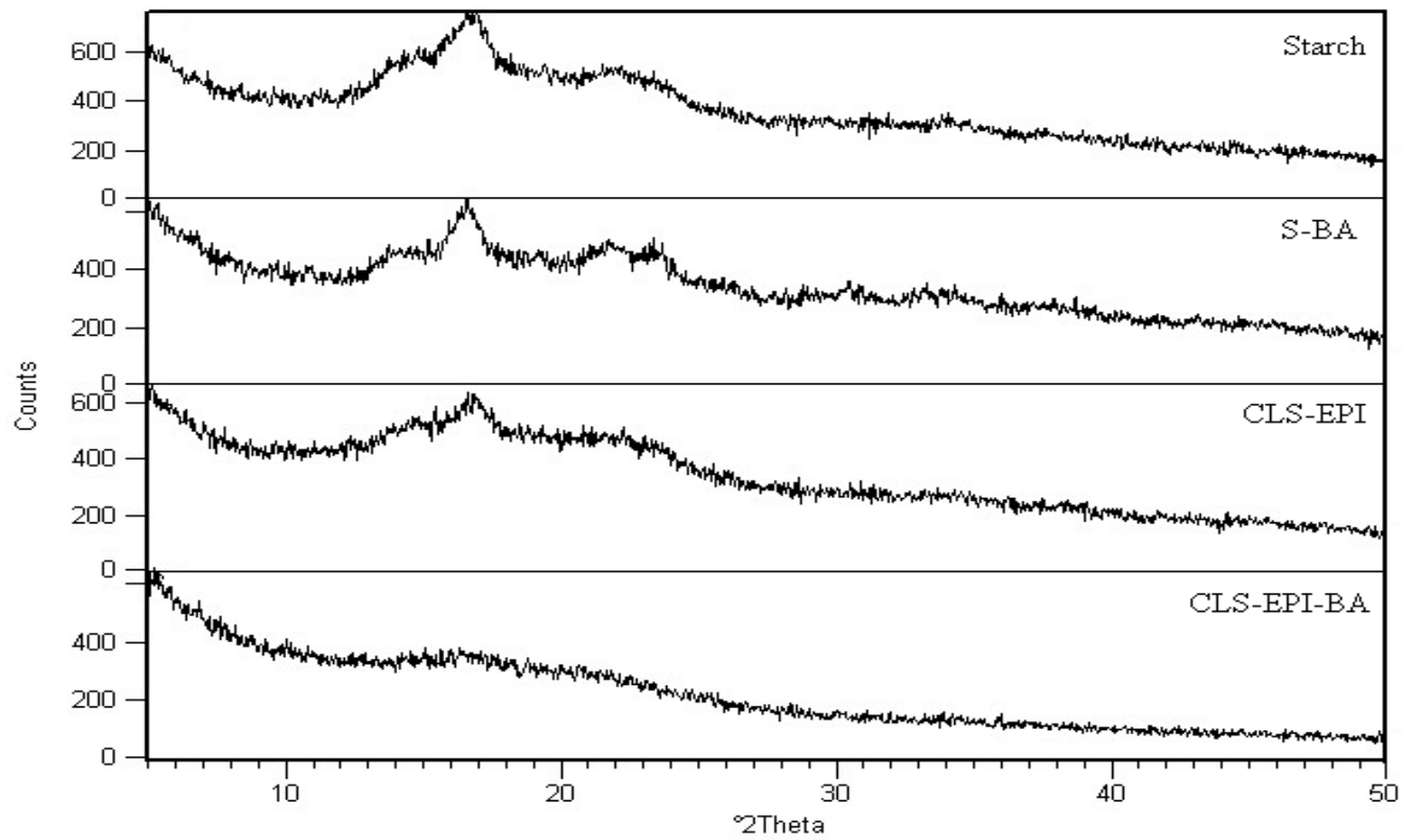


Figure 3.7 The XRD patterns of Starch, S-BA, CLS-EPI and CLS-EPI-BA

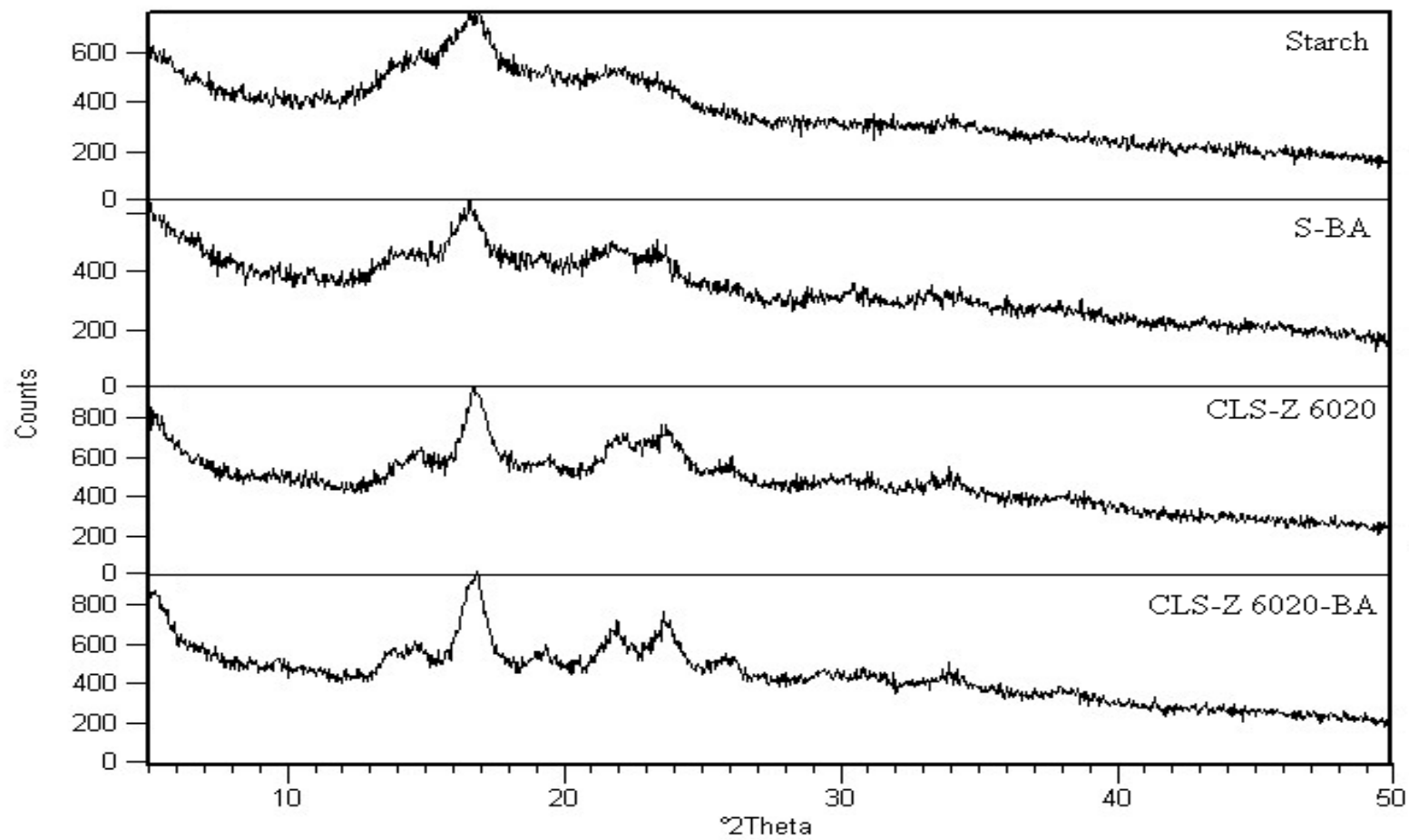


Figure 3.8 The XRD patterns of Starch, S-BA, CLS-Z 6020 and CLS-Z 6020-BA

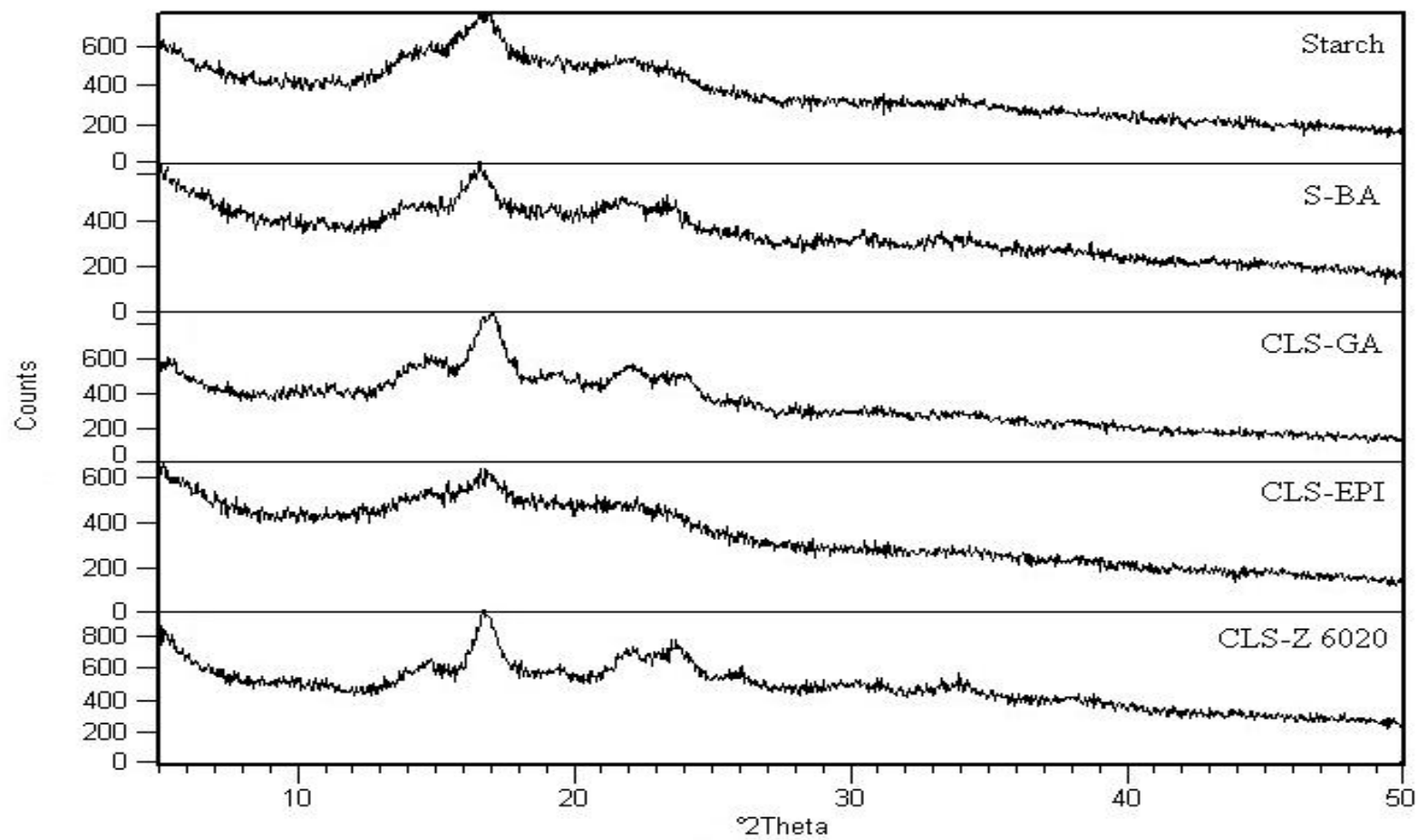


Figure 3.9 Comparison between the XRD patterns of Starch and all cross-linked starch powders

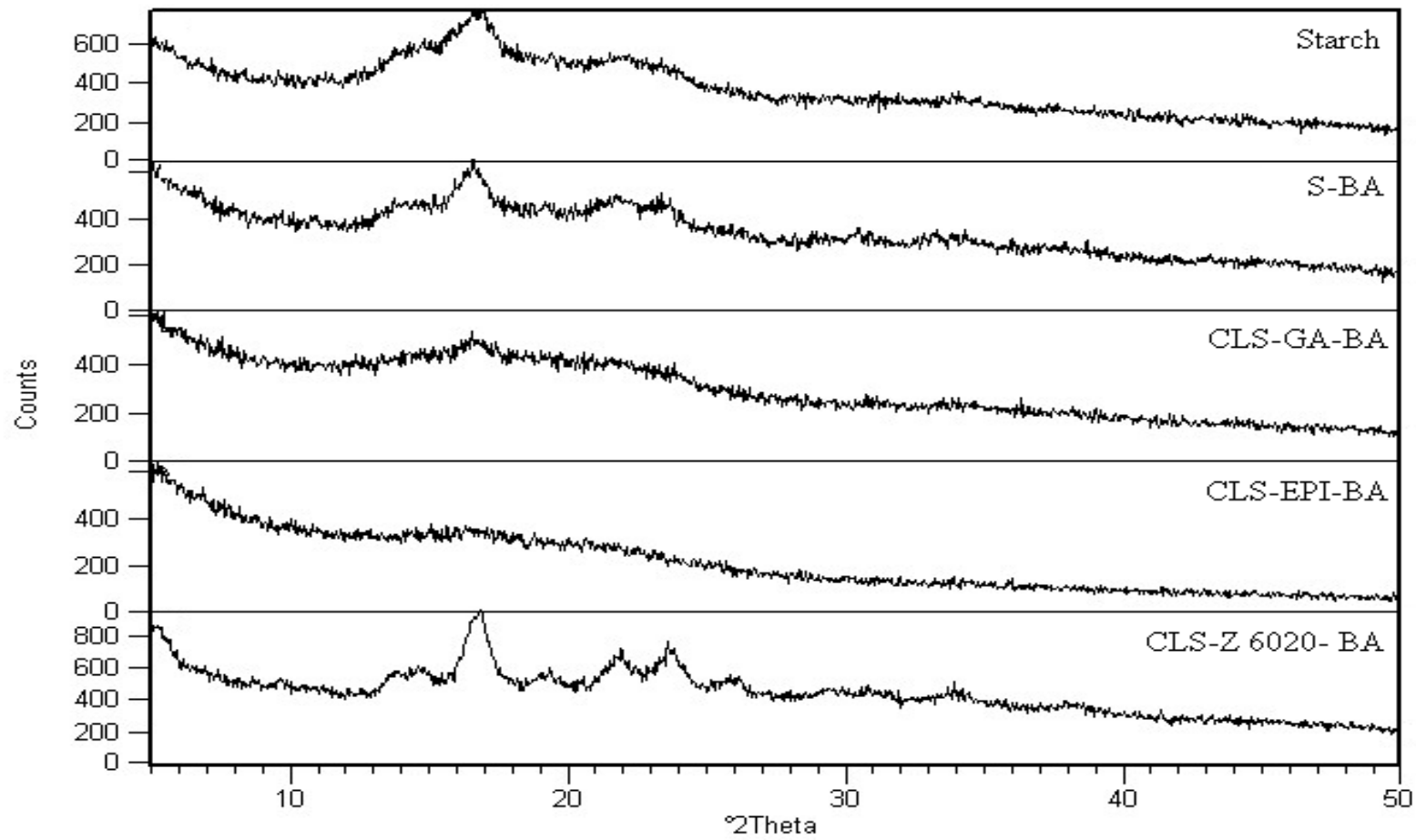


Figure 3.10 Comparison between the XRD patterns of Starch and boron complexes of cross-linked starch powders

Table 3.1 XRD results for synthesized hydrogels

Sample	2 θ (deg)
Starch	16.6
	22.0
PVA	18.9
	39.8
SF-BA	17.1
	19.7
SF-GA	19.6
SF-GA-BA	19.5
SF-EPI	17.0
	19.5
SF-EPI-BA	16.9
	19.4
SF-Z 6020	16.9
	19.5
SF-Z 6020-BA	17.2
	19.6

Table 3.2 XRD results for synthesized powder complexes

Sample	2 θ (deg)
Starch	16.6
	22.0
S-BA	16.6
	21.8
	23.5
CLS-GA	17.1
	22.0
	24.1
CLS-GA-BA	16.6
CLS-EPI	16.9
CLS-EPI-BA	16.3
	16.7
CLS-Z 6020	22.0
	23.7
	16.9
CLS-Z 6020-BA	21.9
	23.7

3.1.2 FTIR analyses

The FTIR spectra of raw materials; starch and PVA are given in Figure 3.11.

An extremely broad band occurs at 3390 cm^{-1} due to hydrogen-bonded hydroxyl groups in the spectrum of starch. Aliphatic C-H stretching vibrations associated with the ring methine hydrogen atoms were observed around 2927 cm^{-1} . The band at 1651 cm^{-1} which was due to water adsorbed in the amorphous regions of starch and the band located at 1372 cm^{-1} was probably related to C-H bending vibrations.

The characteristic absorption bands of starch, which are assigned to C-O stretching vibrations in the C-O-H groups and C-C stretchings, were observed at 1016, 1079 and 1159 cm^{-1} .

Intramolecular and intermolecular hydrogen bondings are expected to occur among PVA chains due to high hydrophilic forces. So, a broad band occurred in the spectrum of PVA at 3428 cm^{-1} which was related to hydrogen bonded -OH functional groups. The bands at 2924 and 2857 cm^{-1} were symmetric and asymmetric C-H stretching vibrations. The aliphatic C-H bending was observed at 1443 cm^{-1} .

The band appeared around 1640 cm^{-1} was attributed to the carbonyl functional groups due to residual acetate groups remaining after the manufacture of PVA from hydrolysis of poly (vinyl acetate) (Jayasekara et al., 2004). The secondary alcoholic C-O stretching absorption takes place at 1090 cm^{-1} .

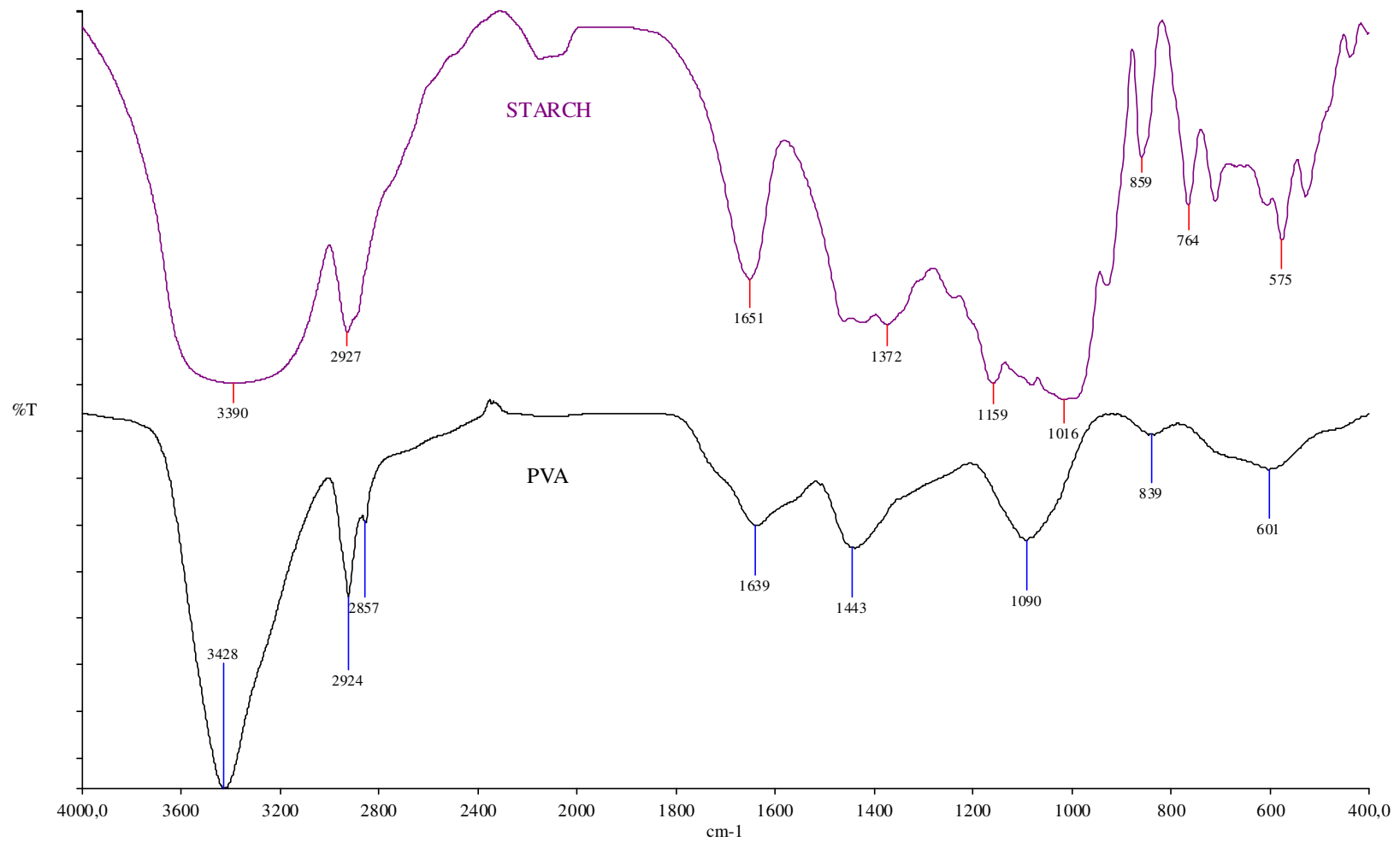


Figure 3.11 The FTIR spectra of starch and PVA

3.1.2.1 FTIR Analyses of Synthesized Hydrogels

FTIR analyses of synthesized hydrogels were shown in Figures 3.12-3.14.

The spectrum of SF-GA (Figure 3.12) showed bands between 3300 and 3000 cm^{-1} indicating the presence of intermolecular and intramolecular hydrogen bonded hydroxyl groups. This gives us the indication of the presence of free hydroxyl groups of both the raw materials in the hydrogel. In addition, the bands at 2971 and 1397 cm^{-1} were attributed to the presence of hydrocarbon chromophore. The bands between 1200-800 cm^{-1} were due to C-O stretchings.

The absence of bands in 1740-1720 cm^{-1} range indicates absence of aldehydic group. From this it can be inferred that all the aldehydic groups of the GA has been used for crosslinking and hence the chance of cytotoxicity is greatly reduced (Pal et al., 2006a).

When BA was used with GA the infrared spectra of the new complex (SF-GA-BA) was slightly changed compared to spectra of SF-GA. Bands which were due to intermolecular and intramolecular hydrogen bonded hydroxyl groups were shifted to higher frequencies while the bands of hydrocarbon chromophore were shifted to lower frequencies. Also the shape of these bands were little bit changed. The intensity of the bands which were related to C-O stretchings in general was increased and these bands were shifted to higher frequencies. When just BA was used (SF-BA), the bands at around 2970 cm^{-1} were shifted to higher frequencies and the intensity of the bands due to C-O stretchings were increased.

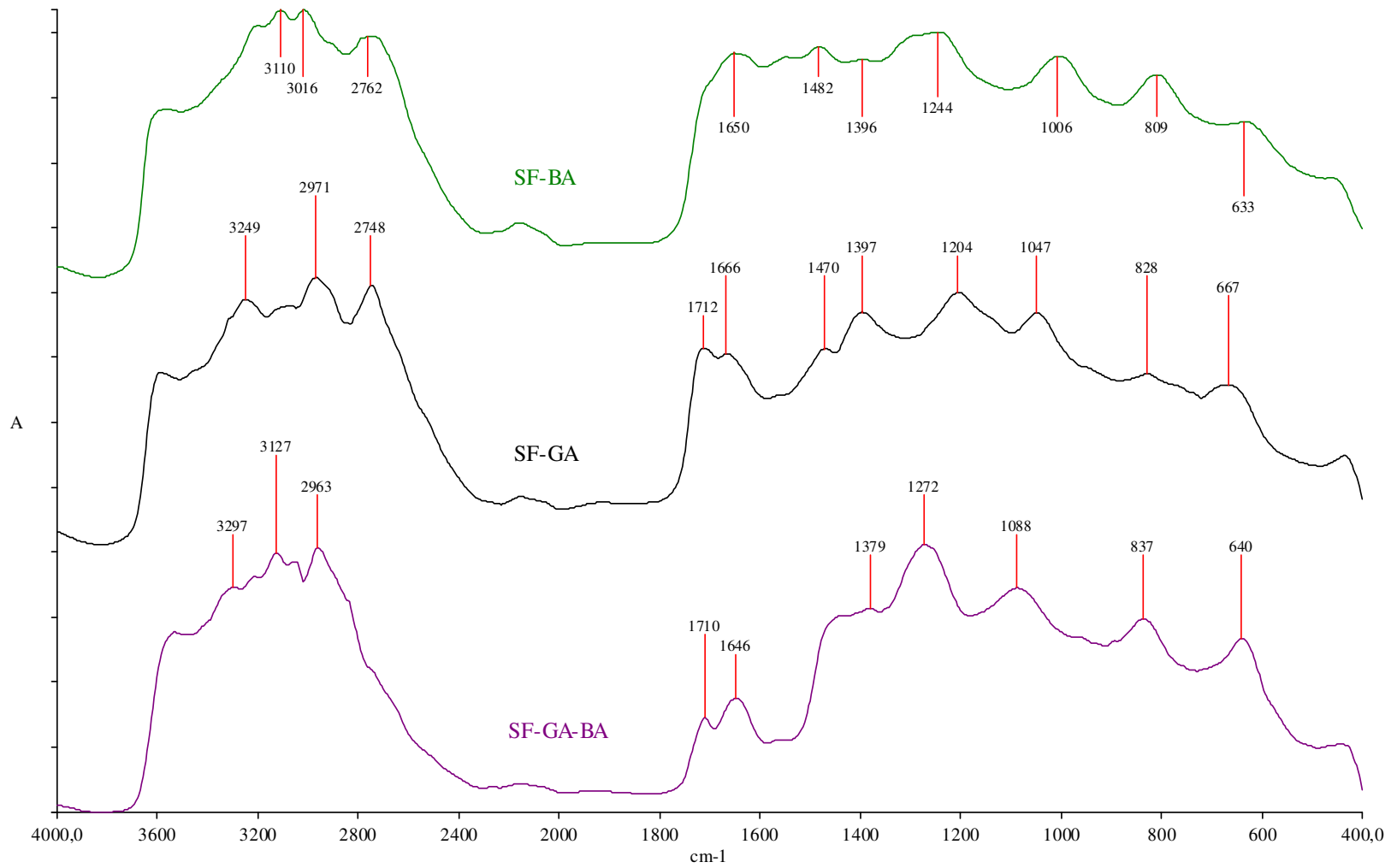


Figure 3.12 The FTIR spectra of hydrogels cross-linked with GA

As starch, PVA and EPI contain bonds including; O-H, C-H, C-O and C-C, the bands in the spectra of hydrogel cross-linked with EPI were similar to those of starch and PVA. In the spectra of SF-EPI (Figure 3.13), the bands of intermolecular and intramolecular hydrogen bonded hydroxyl groups were observed between 3300 and 3000 cm^{-1} . The C-H stretching and bending vibrations located around 2947 and 1371 cm^{-1} , respectively were attributed to the presence of hydrocarbon chromophore. The band around 1645 and 1280 cm^{-1} were due to the water present in amorphous regions of starch and O-H bending, respectively. The bands between 1050-800 cm^{-1} were due to C-O and C-C stretchings.

The intensities of hydrogen bonded hydroxyl groups were considerably decreased where the amount of water present in amorphous regions of starch was increased in the spectrum of both SF-EPI-BA and SF-BA compared to SF-EPI. C-O and C-C bands were a little bit shifted and the intensities of these bands were increased and also the bands of hydrocarbon chromophore were shifted to lower frequencies when EPI and BA were used together for complex formation.

The FTIR spectrum of SF-Z 6020 was given in Figure 3.14. The absorption bands around 3300-3000 cm^{-1} can be considered as O-H stretching vibrations and bands at 2917 and 2794 cm^{-1} were due to C-H stretchings. N-H stretching vibrations of Z-6020 were may be overlapping with O-H stretching bands. The bands around 637 cm^{-1} could be attributed to Si-C bonds. A broad band appeared at 1047 cm^{-1} was may be due to the presence of asymmetric stretching of Si-O-Si bond. This shows that siloxane polymers are formed. Thus the silanes are first hydrolysed and then condensed to oligomers. An absorption band was also observed around 1251 cm^{-1} which corresponds to the Si-O-C bond. Again, the band found around 860 cm^{-1} could also be assigned to Si-OH. The band at 1390 cm^{-1} is possibly from the C-N stretching of amines, while that at 1631 and 1705 cm^{-1} were may due to the NH_2 deformation and 1460 cm^{-1} was due to CH_2 -N stretching.

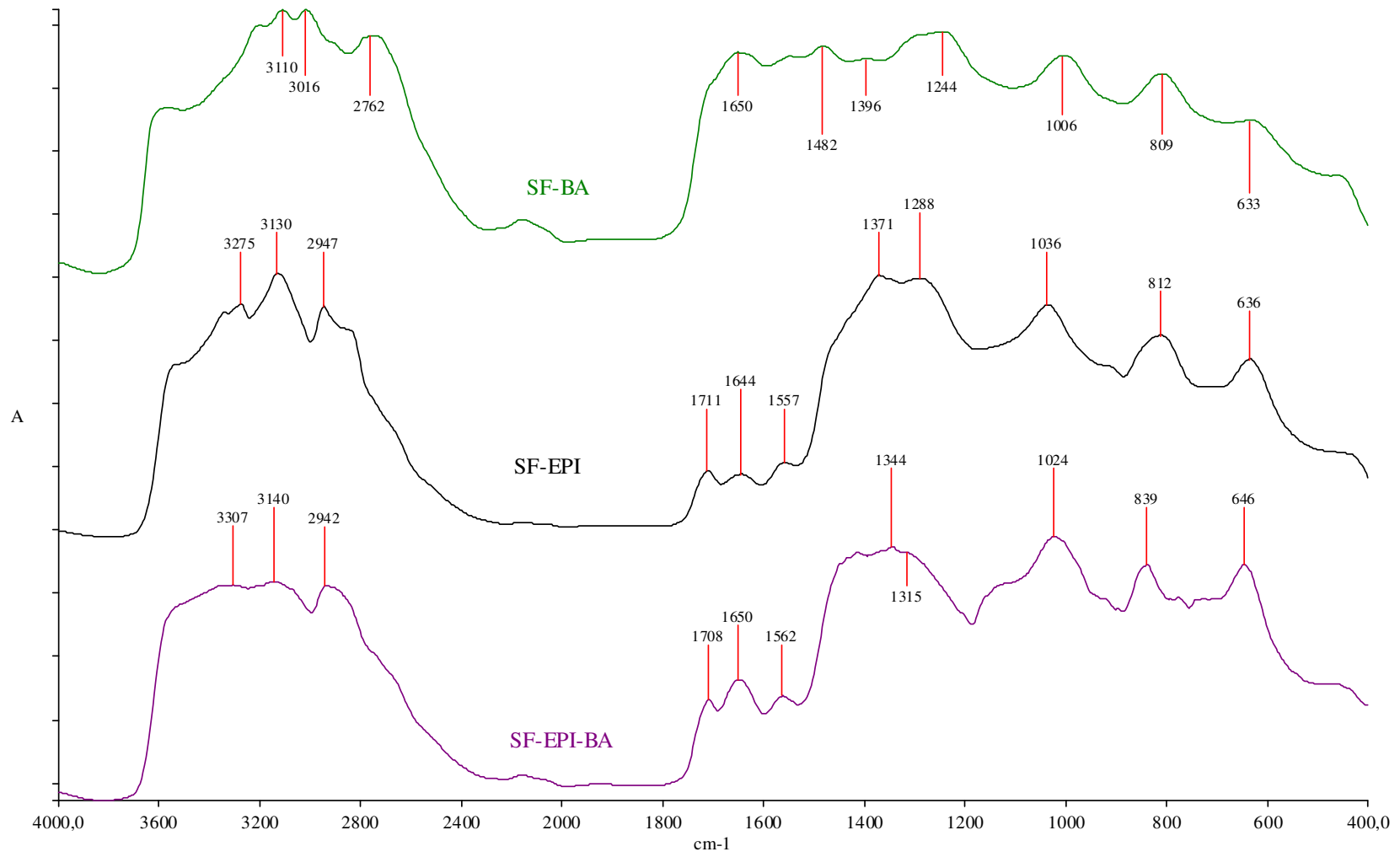


Figure 3.13 The FTIR spectra of hydrogels cross-linked with EPI

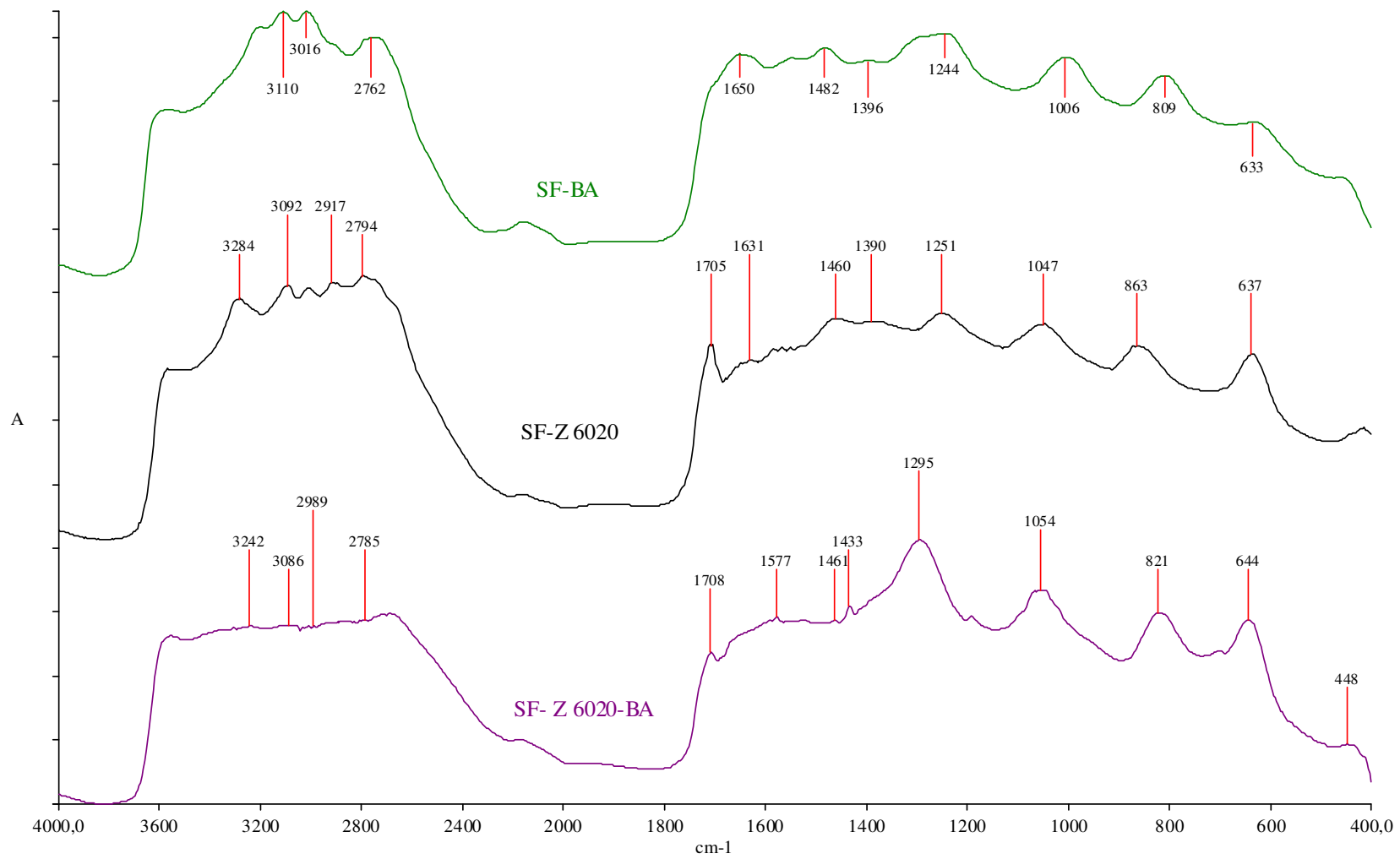


Figure 3.14 The FTIR spectra of hydrogels cross-linked with Z-6020

The intensity of bands related with hydroxyl groups and NH_2 deformations were extremely reduced when BA and Z-6020 were used together in the synthesis of starch/PVA hydrogels compared to the spectrum of SF-Z 6020. However, the bands which were due to Si-O-C and Si-O-Si were shifted to higher frequencies and the intensities of these bands were fairly increased in SF-Z 6020-BA. Si-C band at around 637 cm^{-1} was also shifted to higher frequencies where Si-OH band at 863 cm^{-1} was shifted to lower frequencies in this complex.

Characteristic bands of BA such as B-O stretching and O-B-O bendings could not be distinguished from the other bands of raw materials in the synthesized hydrogels.

3.1.2.2 FTIR Analyses of Synthesized Powder Complexes

The FTIR spectra of starch, BA, cross-linked starches and boron-starch complexes in the powder form were given in Figures 3.15-3.17.

An extremely broad band at 3390 cm^{-1} in the spectra of starch, due to hydrogen-bonded hydroxyl groups was shifted to 3411 , 3409 and 3429 cm^{-1} as a result of cross-linking of starch with GA, EPI and Z-6020, respectively.

Aliphatic C-H stretching vibrations associated with the ring methine hydrogen atoms which were observed around 2927 cm^{-1} and the bands which were due to water adsorbed in the amorphous regions of starch appeared around 1651 cm^{-1} did not almost change with cross-linking reactions. But the band in the spectra of starch located at 1372 cm^{-1} which was probably due to C-H bending vibrations were shifted to higher frequencies after cross-linking for all cross-linkers.

In addition, characteristic absorption bands which were assigned to C-O and C-C stretching vibrations of starch at around 1016 , 1079 and 1159 cm^{-1} , were highly increased in intensity and there were little shifts in these bands after cross-linking procedures.

When BA was used in the synthesis, the bands which were due to B-OH bonds and expected around $3300\text{-}3200\text{ cm}^{-1}$ were overlapped with OH⁻ bands, but in comparison with starch the intensities of these bands were increased and bands were shifted to higher frequencies for S-BA, CLS-GA-BA, CLS-EPI-BA and CLS-Z 6020-BA complexes.

Besides, the characteristic bands related to B-O stretchings around $1500\text{-}1100\text{ cm}^{-1}$, O-B-O bendings around $900\text{-}700\text{ cm}^{-1}$ and B-O deformations around 650 cm^{-1} in the spectrum of BA were rather changed in shape and some of the bands were disappeared or overlapped with some other bands related to starch in the synthesized complexes. From this it can be inferred that BA has been completely used in the complex formation.

The intensities of the bands associated with C-O and C-C stretchings in the spectrum of starch were increased and little shifts were observed in the frequencies of these bands. The bands related with C-H bendings were also shifted to higher frequencies as well as there were minor changes in C-H stretchings for all synthesized boron complexes of starch.

The spectrum of S-BA and the spectrum of CLS-GA-BA, CLS-EPI-BA or CLS-Z 6020-BA were so similar in shape but just the frequencies of most of the bands were quite shifted in the synthesized complexes.

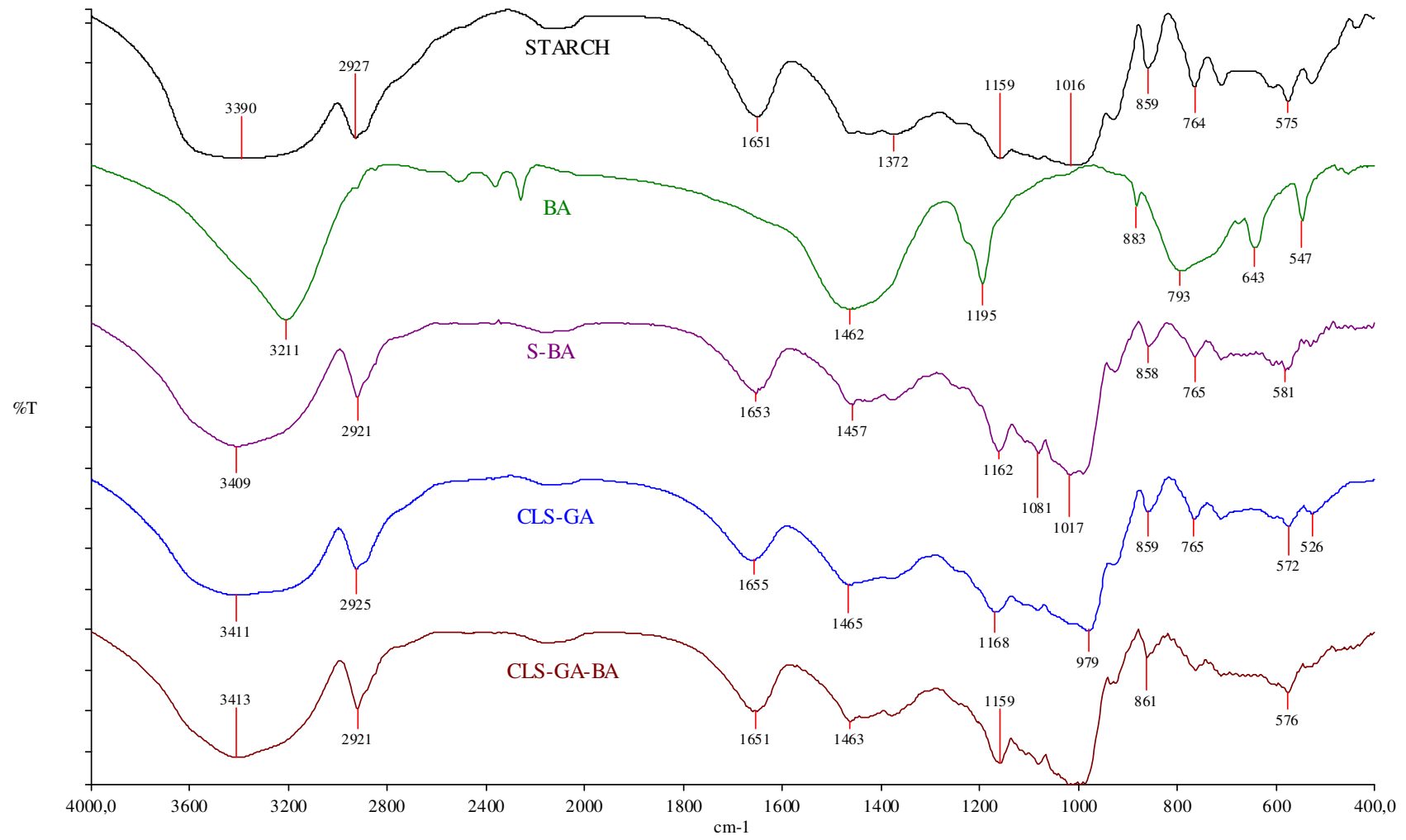


Figure 3.15 The FTIR spectra of powder complexes cross-linked with GA

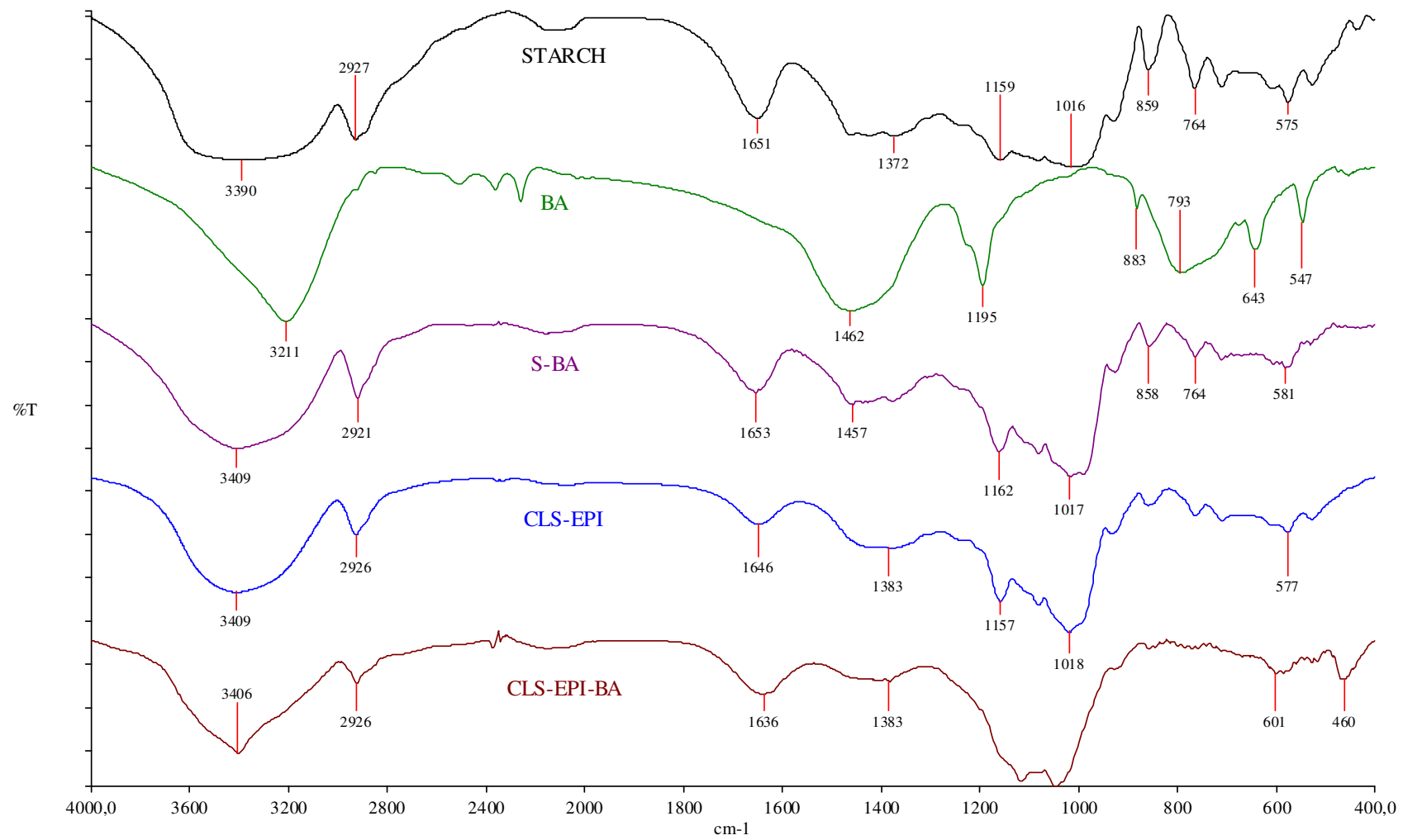


Figure 3.16 The FTIR spectra of powder complexes cross-linked with EPI

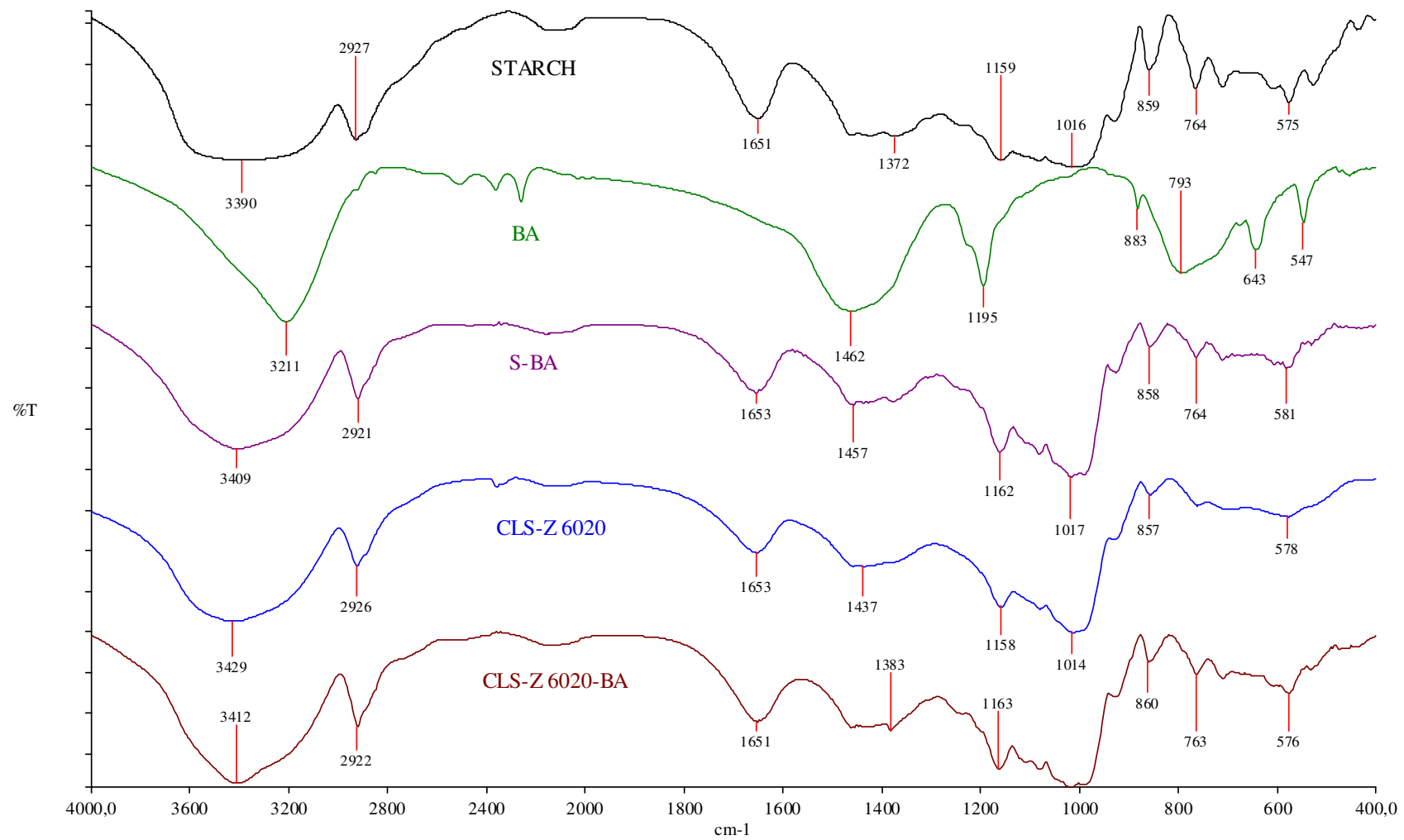


Figure 3.17 The FTIR spectra of powder complexes cross-linked with Z-6020

3.1.3 TGA analyses

3.1.3.1 TGA Analyses of Synthesized Hydrogels

TGA is based on continuous measurements of weight on a sensitive balance (microbalance) as the sample temperature is increased in an inert atmosphere.

The TGA thermograms and derivative thermogravimetric (DTG) curves of starch, PVA and all synthesized hydrogels were presented in Figures 3.18-3.21. The thermogram of starch showed two-step decomposition while PVA and synthesized hydrogels were exhibiting three-step thermal decomposition. The first step of mass loss could be attributed to evaporation of adsorbed water for all samples. The other steps were due to thermal decomposition stages of the products.

The shoulder in the starch degradation peak that was observed in the second step may have been due to the different degradation rates of amylose and amylopectin, the former probably being degraded first because of its linear structure (Sreedhar et al., 2005).

The thermogravimetric data (TGA and DTG) of all hydrogel samples were given in Table 3.3. Evaporation of adsorbed water was carried out between 110-125 °C with 3-9% mass loss for hydrogels and thermal decompositions of the hydrogels were realized above 300 °C and 400 °C with average 58% and 20% mass losses for second and third steps, respectively.

It is concluded that the cross-linking of starch/PVA hydrogels lead to the formation of more thermally stable copolymers than starch and PVA. The use of BA in the synthesis of the hydrogels improves the thermal stability of starch and PVA more than cross-linked starch/PVA hydrogels.

Table 3.3 Thermogravimetric (TGA and DTG) data* of starch, PVA and synthesized hydrogels

SAMPLE	FIRST STEP		SECOND STEP		THIRD STEP	
	DTG Maxima (°C)	% Mass loss	DTG Maxima (°C)	% Mass loss	DTG Maxima (°C)	% Mass loss
STARCH	55	7	299	76	-	-
PVA	25	4	267	67	418	22
SF-GA	112	9	313	61	419	22
SF-EPI	112	6	330	59	423	22
SF-Z 6020	123	7	311	61	424	16
SF-BA	114	8	308	62	420	17
SF-GA-BA	111	7	341	59	423	22
SF-EPI-BA	114	3	336	54	432	20
SF-Z 6020-BA	120	5	313	53	430	19

* Data were obtained under dynamic nitrogen atmosphere at a heating rate of 10°C/min

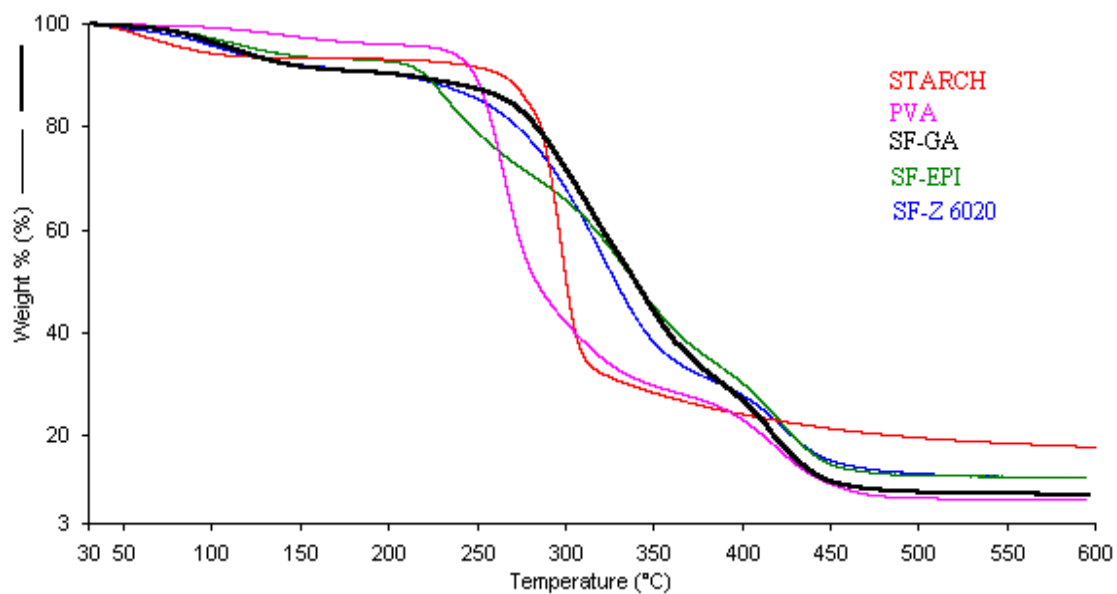


Figure 3.18 TGA curves of starch, PVA and synthesized hydrogels

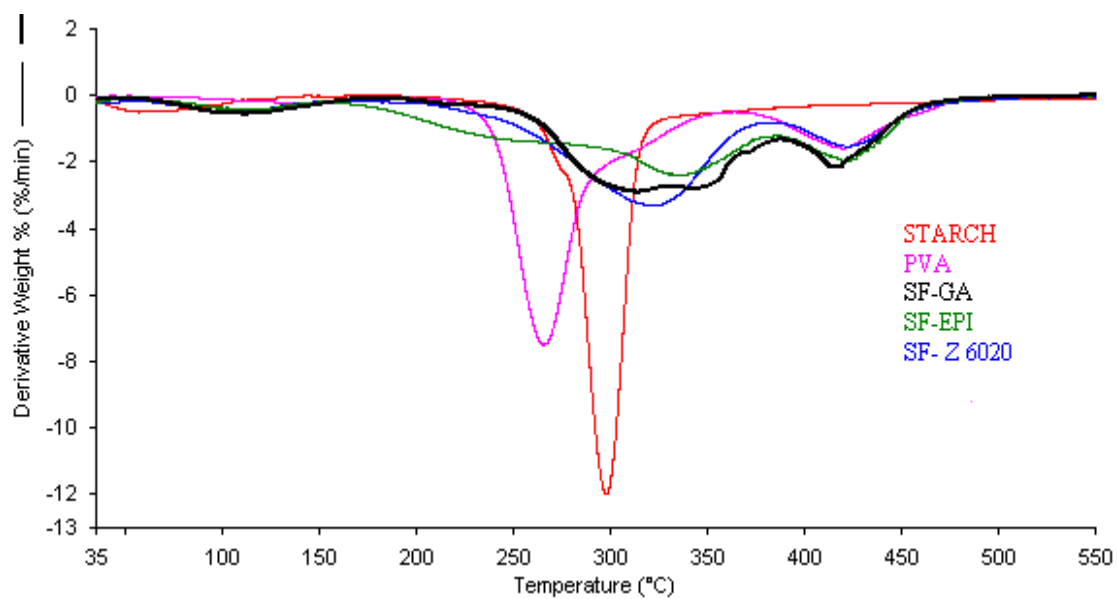


Figure 3.19 DTG curves of starch, PVA and synthesized hydrogels

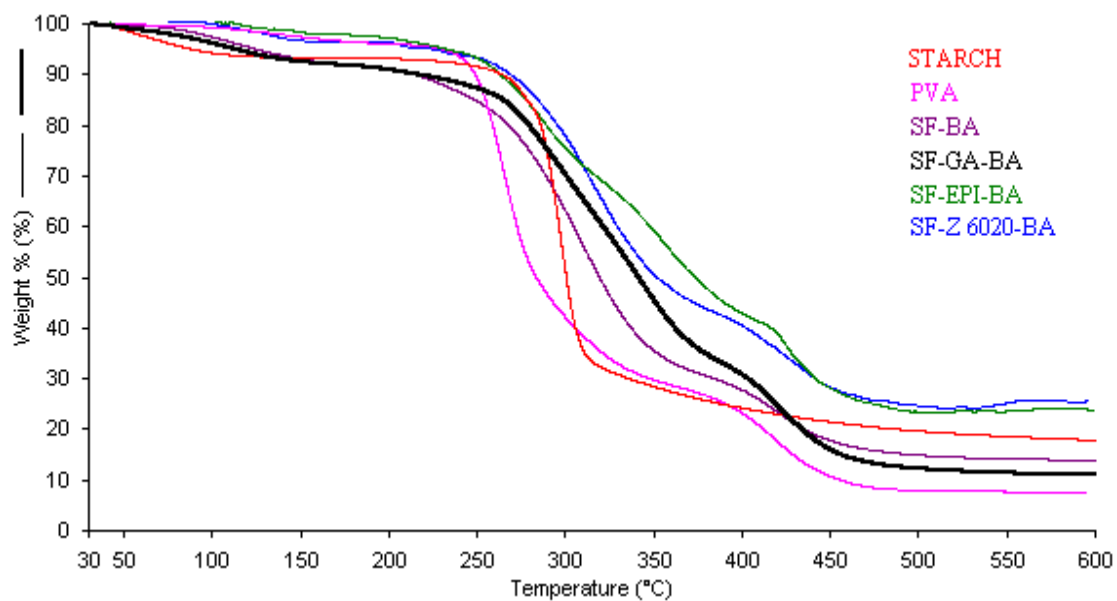


Figure 3.20 TGA curves of starch, PVA and boron-starch/PVA hydrogels

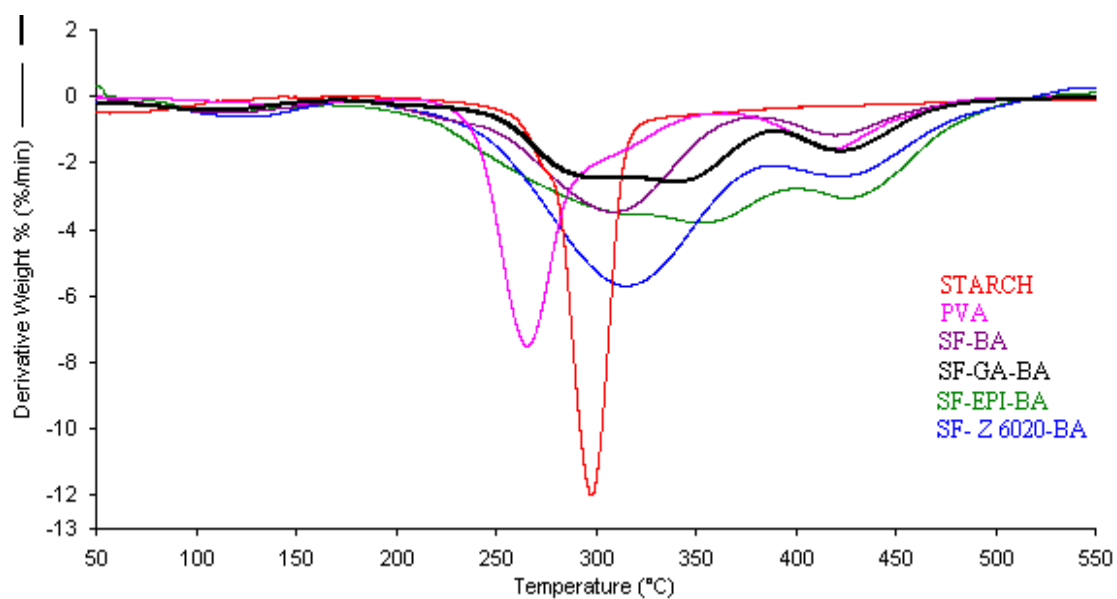


Figure 3.21 DTG curves of starch, PVA and boron-starch/PVA hydrogels

3.1.3.2 TGA Analyses of Synthesized Powder Complexes

Figures 3.22-3.25 were exhibiting TGA and DTG curves for starch, cross-linked starches and boron complexes of starch in the powder form.

As can be seen there were two significant decomposition steps for starch and all other synthesized powder complexes. The first step of which DTG maxima were between 46 and 63 °C could be attributed to evaporation of adsorbed water and the mass losses were obtained to be maximum 12% for starch and all powder complexes. The other decomposition steps of which DTG maxima were between 276 and 299 °C; were due to thermal decomposition of starch, cross-linked starches and boron complexes and the total mass losses were appeared approximately between 65-80% for powder complexes.

The thermogravimetric data of all powder samples were given in Table 3.4. The results denoted that, the adsorbed water was evaporated at higher temperatures for powder complexes except CLS-GA, while thermal decompositions of synthesized complexes were occurred at few lower temperatures compared with starch.

This means, unlike hydrogels thermal stability of starch was decreased for both cross-linked starches and boron complexes of starch after modification process. The amount of cross-linking agents used in the synthesis of powder complexes was less than hydrogels so the result was acceptable for powder complexes. Because, type and concentration of the reagent used for cross-linking significantly affects the extent of change in thermal properties (Singh et al., 2007).

Table 3.4 Thermogravimetric (TGA and DTG) data* of starch, cross-linked starch powders (CLS) and boron complexes of starch

SAMPLE	FIRST STEP		SECOND STEP	
	DTG Maxima (°C)	% Mass loss	DTG Maxima (°C)	% Mass loss
STARCH	55	7	299	76
CLS-GA	46	10	288	70
CLS-EPI	56	5	297	77
CLS-Z 6020	57	6	289	71
S-BA	63	12	289	67
CLS-GA-BA	62	11	276	66
CLS-EPI-BA	61	8	298	67
CLS-Z 6020-BA	62	8	285	80

* Data were obtained under dynamic nitrogen atmosphere at a heating rate of 10°C/min

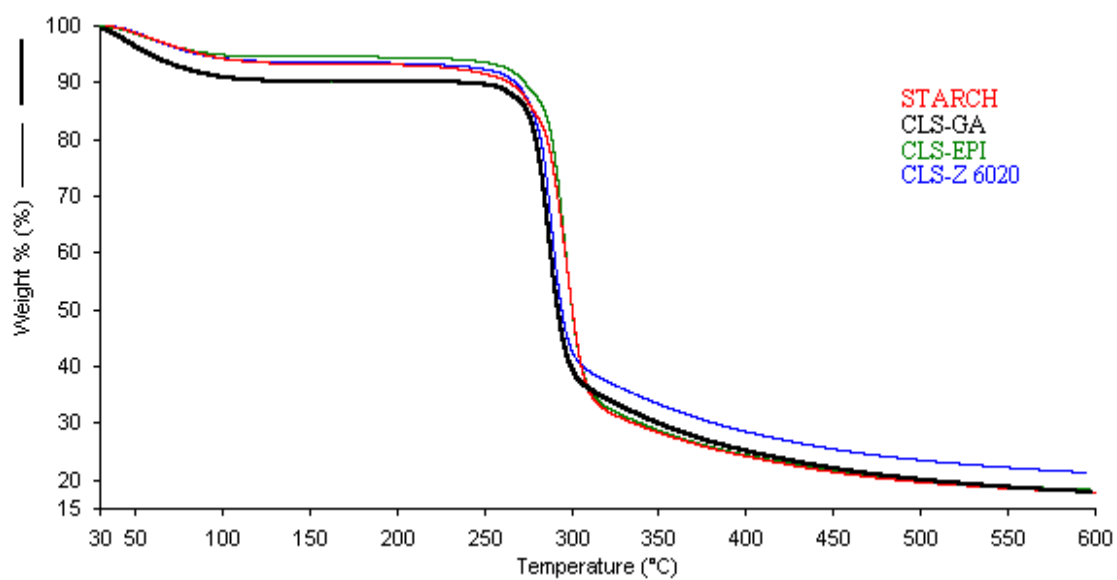


Figure 3.22 TGA curves of starch and cross-linked starch powders

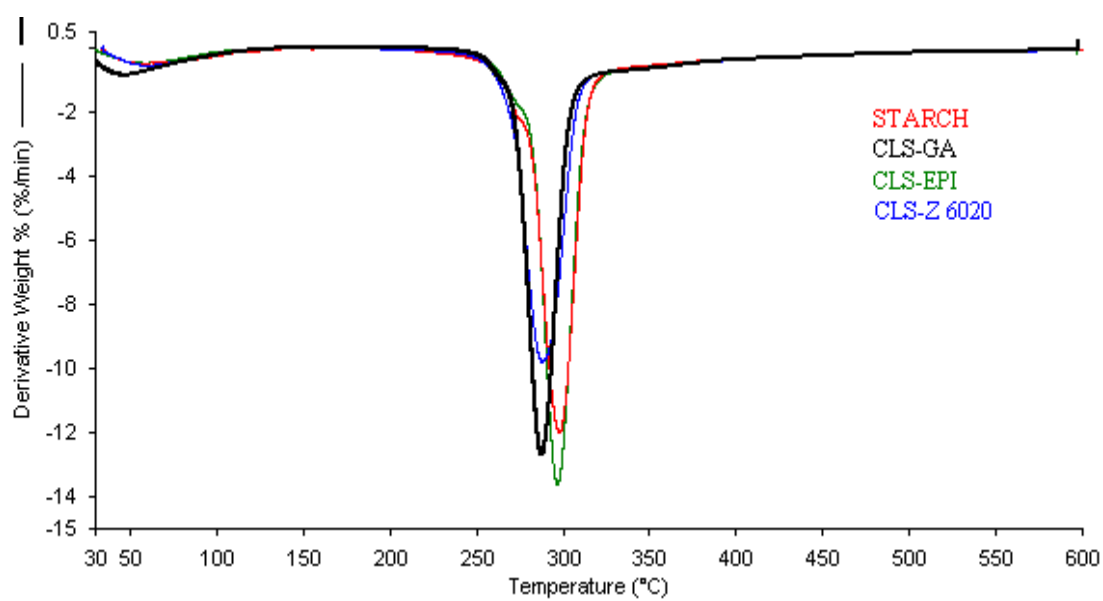


Figure 3.23 DTG curves of starch and cross-linked starch powders

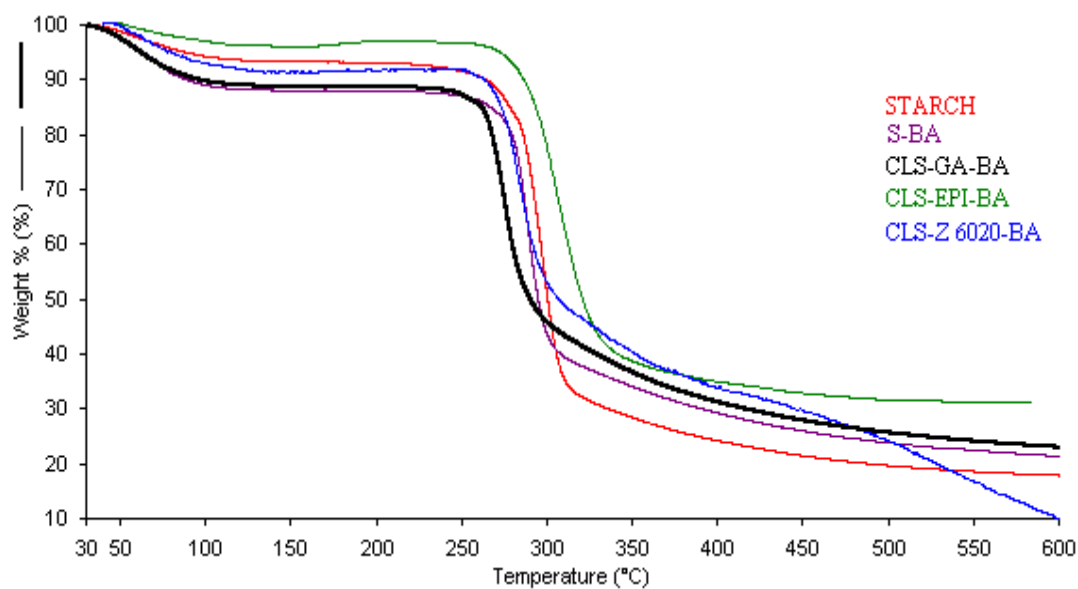


Figure 3.24 TGA curves of starch and boron complexes of starch

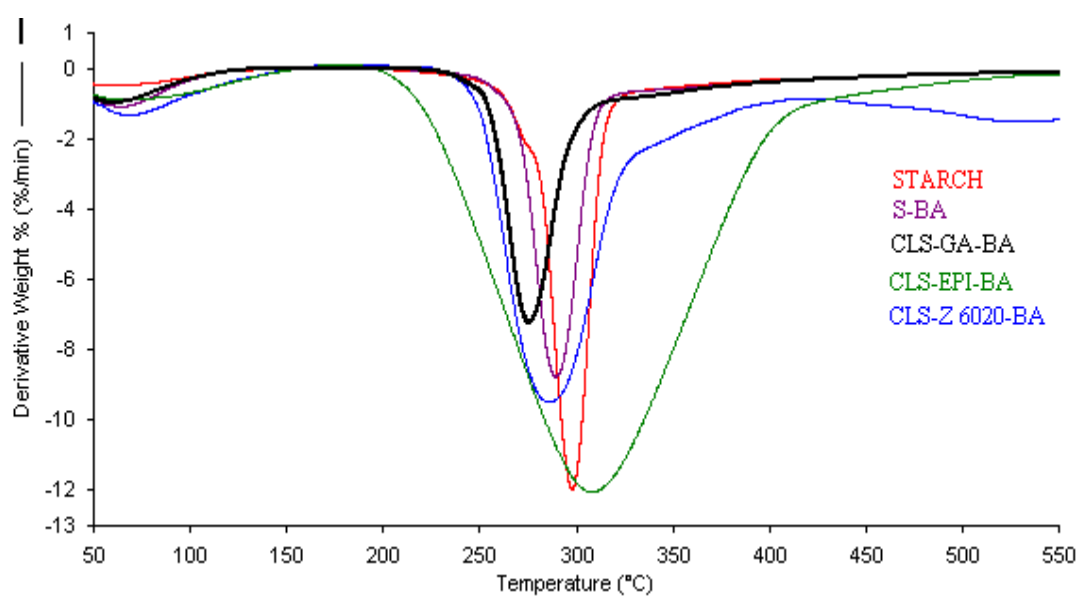


Figure 3.25 DTG curves of starch and boron complexes of starch

3.1.4 SEM analyses

SEM analysis has been applied to synthesized boron-starch complexes and boron-starch/PVA hydrogels. The photos of the surface of the complexes at x300 magnification are shown in Figures 3.26-3.32.

The size and shape of starch particles depend on the source of starch. The particles could vary in size from about 5 microns for rice starch up to 100 microns for potato starch. The shape of the starch particle will also vary depending on the plant source. Starch could be spherical and oval as with potato starch, truncated and round as with tapioca starch, angular and polygonal as with rice starch, and round and polygonal as with corn starch (Rutenberg, 1980).

When the morphology of the raw materials used in this study were investigated; the potato starch exhibited good oval shaped sphericity and a smooth surface (Figure 3.26a), besides PVA has a rough surface (Figure 3.26b) as shown by scanning electron microscope. Also, BA (Figure 3.26c) was observed like spherical shaped particles with a diameter of approximately 400 μm .

3.1.4.1 SEM Analyses of Synthesized Hydrogels

All cross-linked starch/PVA hydrogels revealed an irregular morphology. The SEM analysis results showed that the morphology of starch was changed when starch was blended with PVA in the synthesized hydrogels as already described and demonstrated with XRD analysis.

There were holes and pores inside the materials. Especially boron complex of starch/PVA (SF-BA) had excessive amount of holes (Figure 3.27a) compared to cross-linked starch/PVA hydrogels (Figure 3.27b, 3.28b and 3.29b). There were some deformed spheres of starch found at the surface of SF-BA indicating the irregular and incompact structure of the complex.

This situation showed us that, BA by itself was insufficient for the cross-linking of starch with PVA compared to other cross-linking agents.

The size of the holes were less in SF-GA among the other cross-linked hydrogels, may be due to the high degree of cross-linking, so a compact and porous structure was observed for this hydrogel. The absence of rough scenes encountered in SF-EPI and SF-Z 6020 is also remarkable for SF-GA.

Surface morphology of boron-starch /PVA hydrogels showed that regular shaped spheres were seen when BA was used with cross-linking agents in the synthesis and the size of the spheres decreased in the order of SF-GA-BA (Figure 3.27c), SF-Z 6020-BA (Figure 3.29c) and SF-EPI-BA (Figure 3.28c). Even, spheres were seen like blisters in SF-EPI-BA.

3.1.4.2 SEM Analyses of Synthesized Powder Complexes

According to SEM images of the boron-starch powder complexes, it could be claimed that when starch was treated with BA (Figure 3.31a) or EPI (Figure 3.31b), big aggregates were formed. Besides GA (Figure 3.30b) did not give any aggregates and Z-6020 (Figure 3.32b) gave just small ones.

When BA and cross-linkers were used together in the synthesis the aggregates of S-BA were dissociated into smaller particles (Figure 3.30c, 3.31c and 3.32c).

After being cross-linked, potato starch granules remain smooth and similar to native starch granules in morphology when viewed under SEM, suggesting that the modification does not cause any detectable morphological change (Kaur et al., 2006).

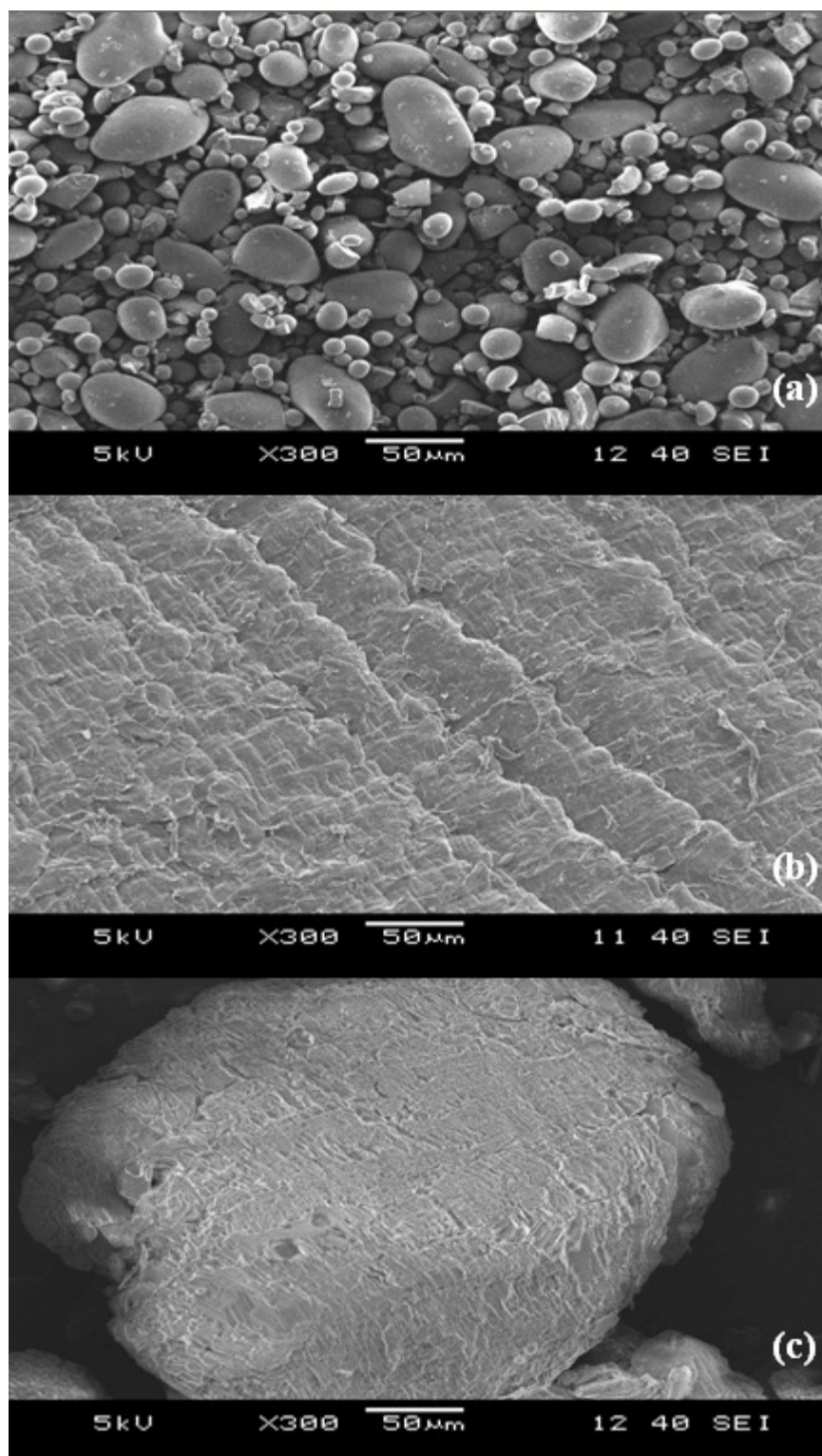


Figure 3.26 SEM photographs of the surface of raw materials at x300 magnification. (a) Starch, (b) PVA, (c) BA

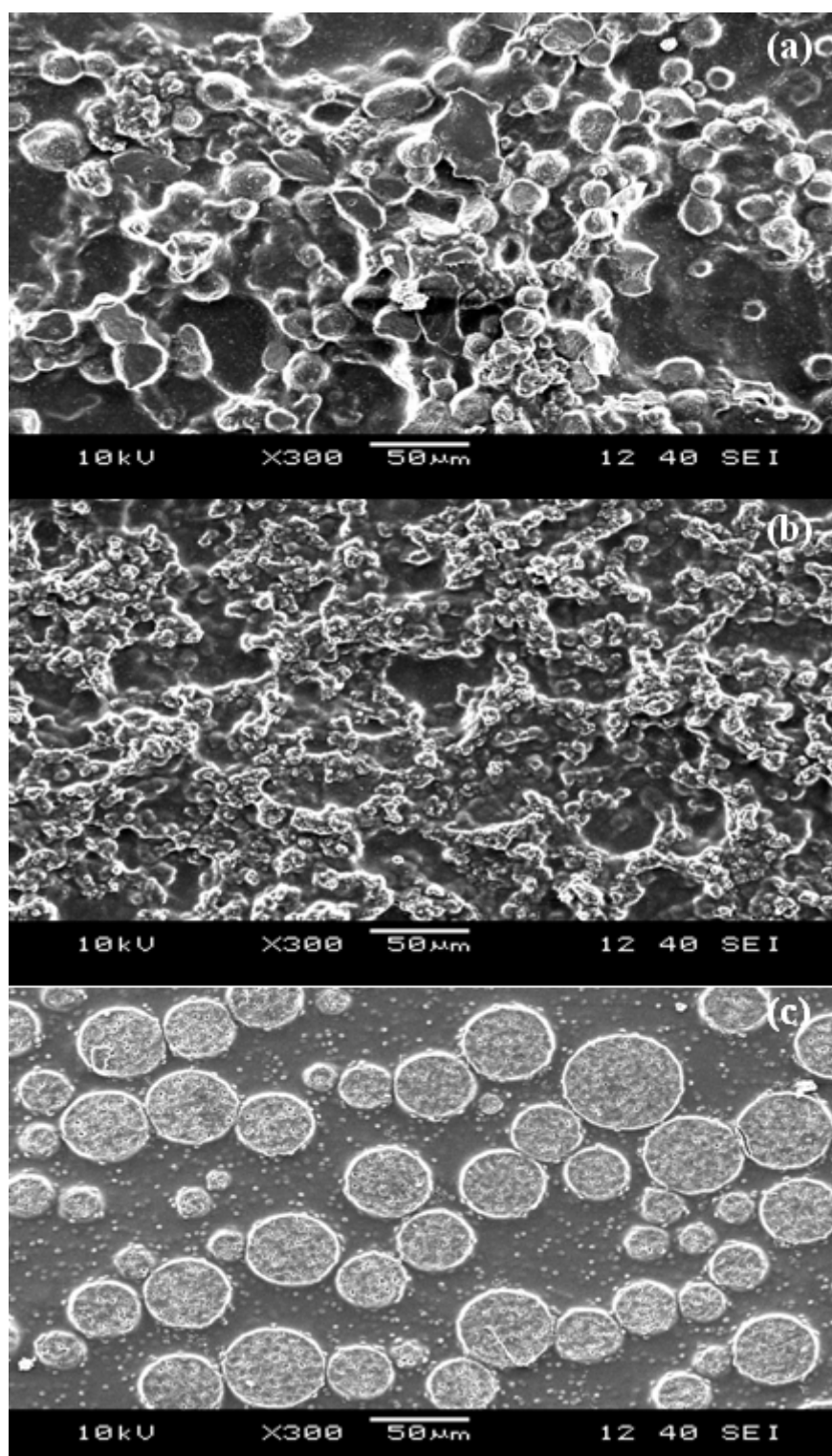


Figure 3.27 SEM photographs of the surface of boron-starch/PVA hydrogels at x300 magnification. (a) SF-BA, (b) SF-GA, (c) SF-GA-BA

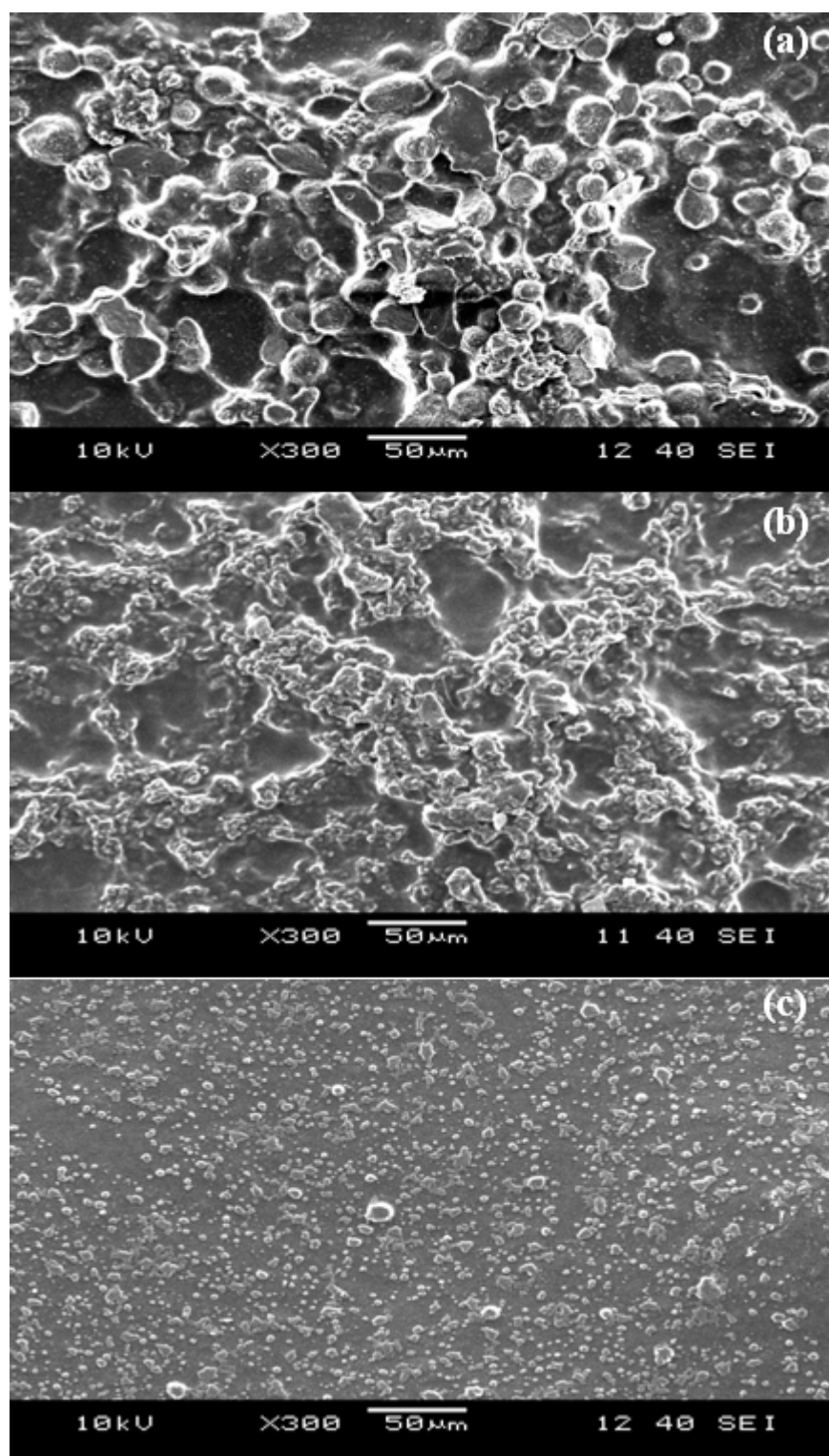


Figure 3.28 SEM photographs of the surface of boron-starch/PVA hydrogels at x300 magnification. (a) SF-BA, (b) SF-EPI, (c) SF-EPI-BA

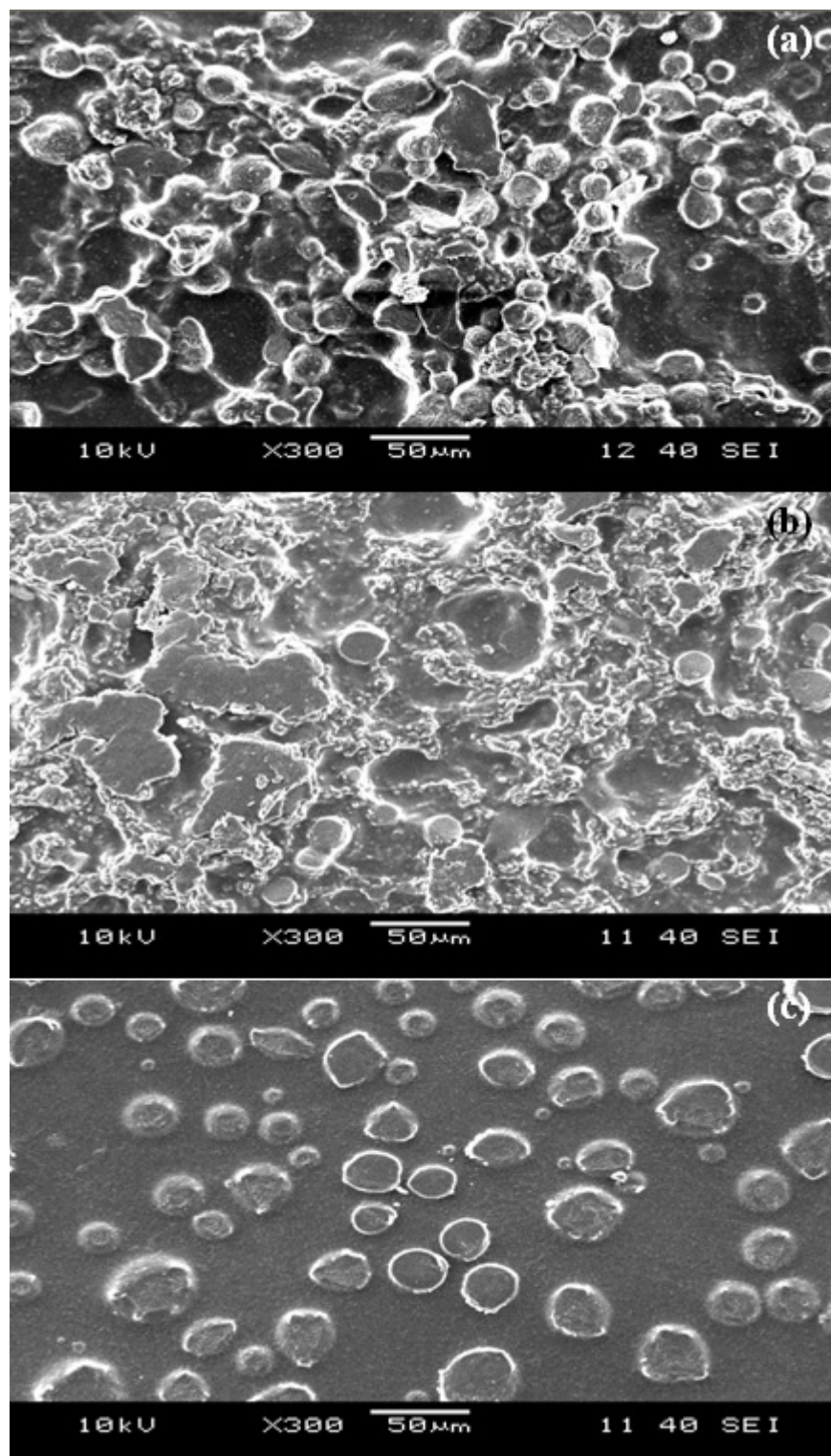


Figure 3.29 SEM photographs of the surface of boron-starch/PVA hydrogels at x300 magnification. (a) SF-BA, (b) SF-Z 6020, (c) SF-Z 6020-BA

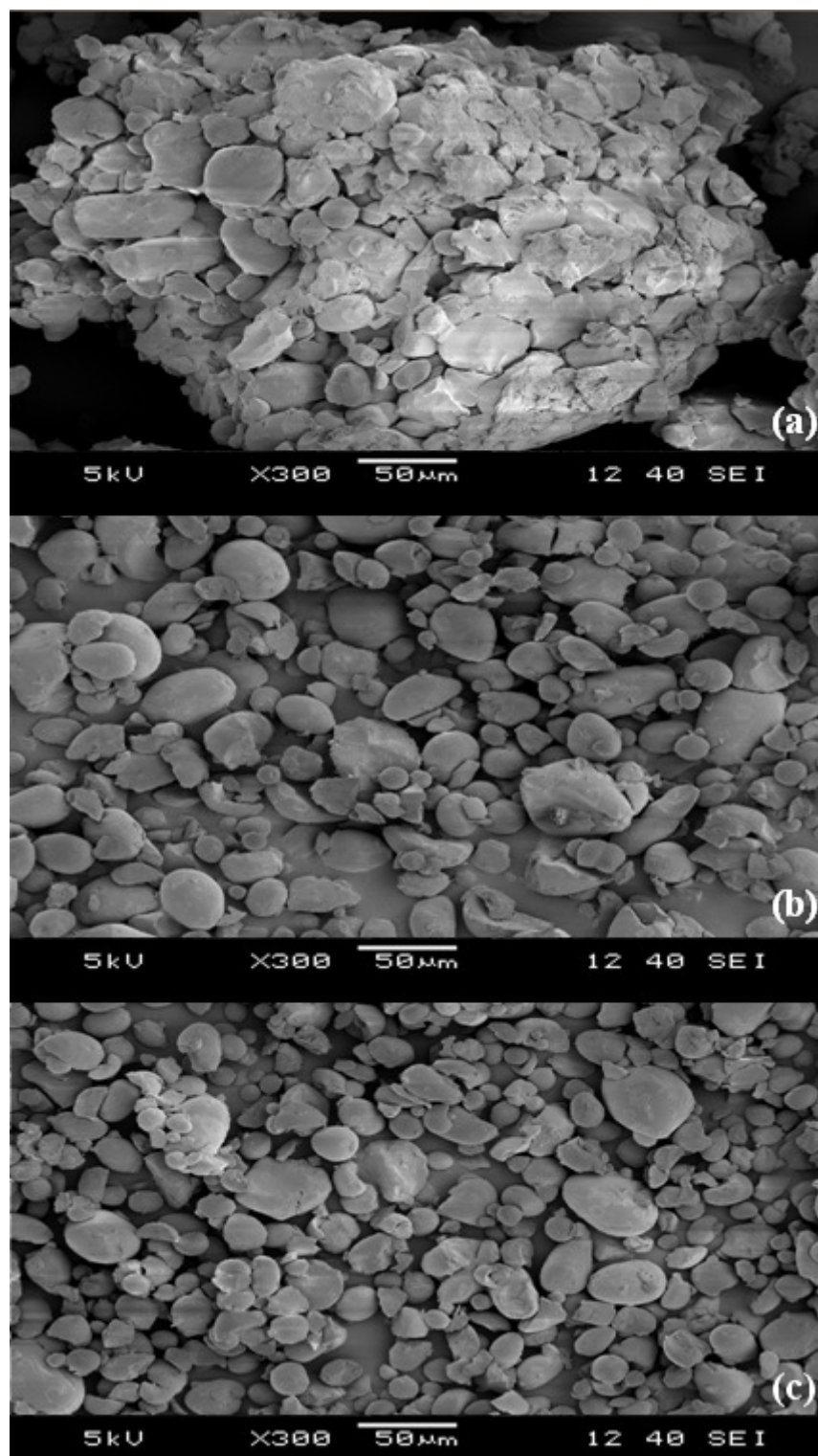


Figure 3.30 SEM photographs of the surface of boron-starch complexes at x300 magnification. (a) S-BA, (b) CLS-GA, (c) CLS-GA-BA

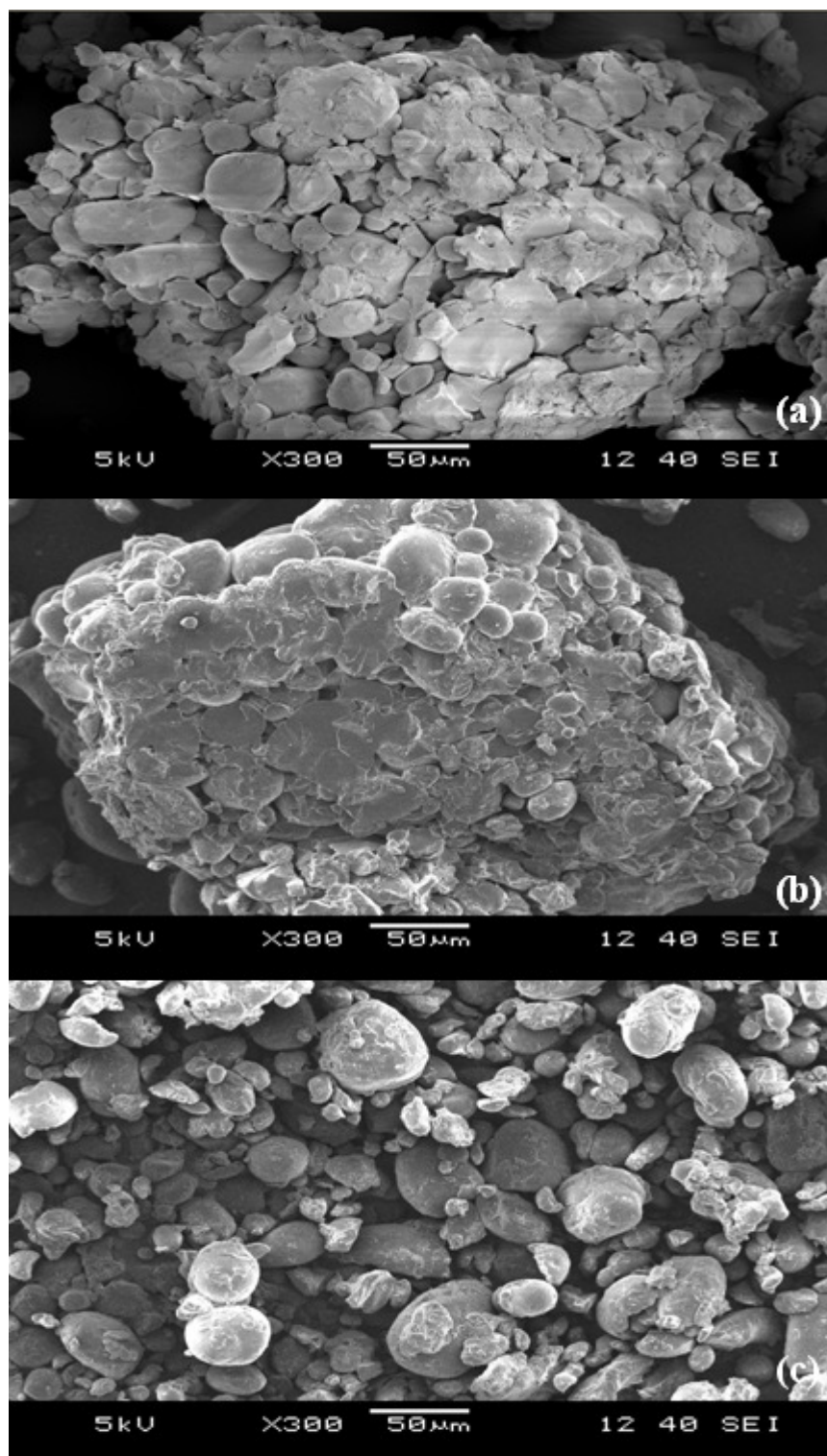


Figure 3.31 SEM photographs of the surface of boron-starch complexes at x300 magnification. (a) S-BA, (b) CLS-EPI, (c) CLS-EPI-BA

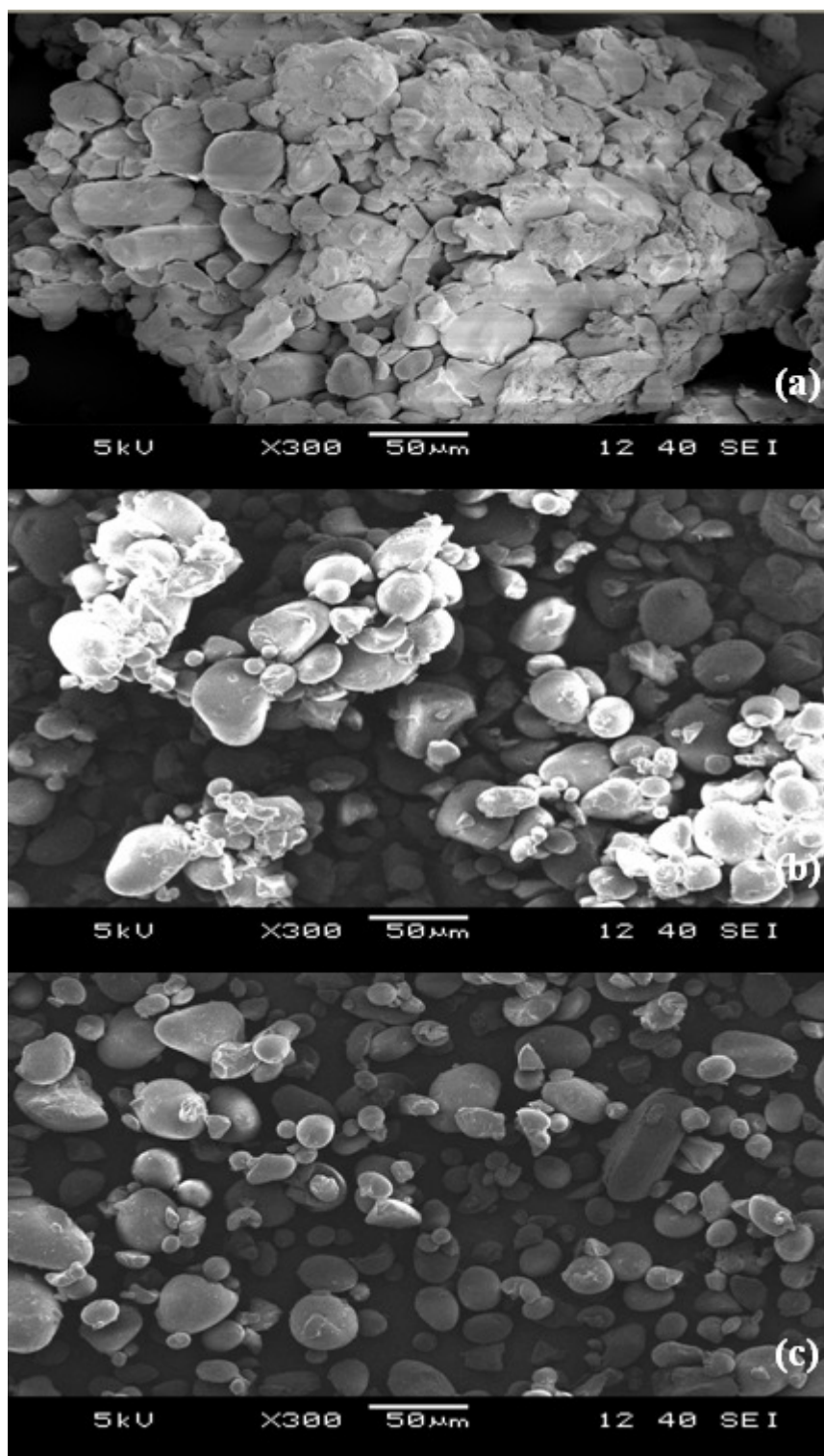


Figure 3.32 SEM photographs of the surface of boron-starch complexes at x300 magnification. (a) S-BA, (b) CLS-Z 6020, (c) CLS-Z 6020-BA

3.1.5 Mechanical Properties of the Synthesized Hydrogels

The mechanical properties of starch/PVA hydrogels were measured and the results were summarized in Table 3.5.

T-test was performed to compare the experimental data and statistical significance was set at 0.05. According to statistical analysis, it was found that the difference was not statistically significant ($p > 0.05$) among the TS, E and $\epsilon\%$ of the hydrogels.

The TS values of SF-BA, SF-GA, SF-GA-BA, SF-EPI, SF-EPI-BA, SF-Z6020 and SF-Z6020-BA were found; 47.39, 43.62, 50.15, 44.92, 44.01, 38.26 and 42.99 MPa, respectively. The alteration of E expressed a tendency similar to that of the TS for the synthesized hydrogels. The breaking elongation values were between 3.85%-5.94% for all samples.

Table 3.5 Mechanical properties of the starch/PVA hydrogels

Samples	Tensile Strength (MPa)	Young's Modulus (GPa)	Elongation at break (%)
SF-BA	47.39	1.70	4.95
SF-GA	43.62	1.54	3.85
SF-GA-BA	50.15	1.81	5.94
SF-EPI	44.92	1.56	4.53
SF-EPI-BA	44.01	1.50	4.06
SF-Z 6020	38.26	1.26	4.07
SF-Z 6020-BA	42.99	1.33	4.44

3.2 pH Dependent Swelling Behaviors of Synthesized Hydrogels

The results of equilibrium swelling times of the hydrogels at ultra pure water and citric acid-phosphate solutions of definite pH values were represented in Figures 3.33-3.37.

The equilibrium times were found to be approximately 30 min for SF-BA and SF-GA-BA, 45 min for SF-EPI-BA and SF-Z 6020-BA, 60 min for SF-EPI and SF-Z 6020, and 180 min for SF-GA at water and at each pH solution. It was seen that swelling equilibrium times were shorter for the boron containing complexes.

Swelling percentages were calculated between 200-300% for cross-linked complexes and 300-500% for boron complexes of starch/PVA hydrogels except SF-GA-BA with a minimum swelling ratio of 86%.

Therefore, it could be suggested that the major cross-linking was occurred in SF-GA-BA, because it was known that cross-linking causes the granules to become compact and absorb less water than the native starch so decreased the swelling power.

When BA was used for complex formation together with EPI and Z-6020 or just by itself, proper compact structure which was seen in SF-GA-BA or in cross-linked starch/PVA hydrogels could not be achieved, thereby swelling ratios were increased.

Because maximum swelling time was found 3 hours for one of the synthesized hydrogels, membranes were kept in water and in all of the solutions of definite pH values for 3 hours while investigating the pH dependent swelling behaviors.

During the pH dependent swelling experiments the hydrogels were swollen approximately 350%, 275%, 255%, 275%, 90%, 450% and 350% at water for SF-BA, SF-GA, SF-EPI, SF-Z 6020, SF-GA-BA, SF-EPI-BA and SF-Z 6020-BA respectively.

When the same samples were immersed in the citric acid-phosphate solutions of different pH values and leaved in each solution for maximum 3 hours no remarkable change was observed in the swelling ratio for all hydrogels which was shown in Figure 3.37.

These results denoted that the swelling behavior of the membranes were essentially independent of pH and were based on water. As starch and PVA contained no ionizable functional groups (Kim & Peppas, 2002), this result was compatible with the expected one.

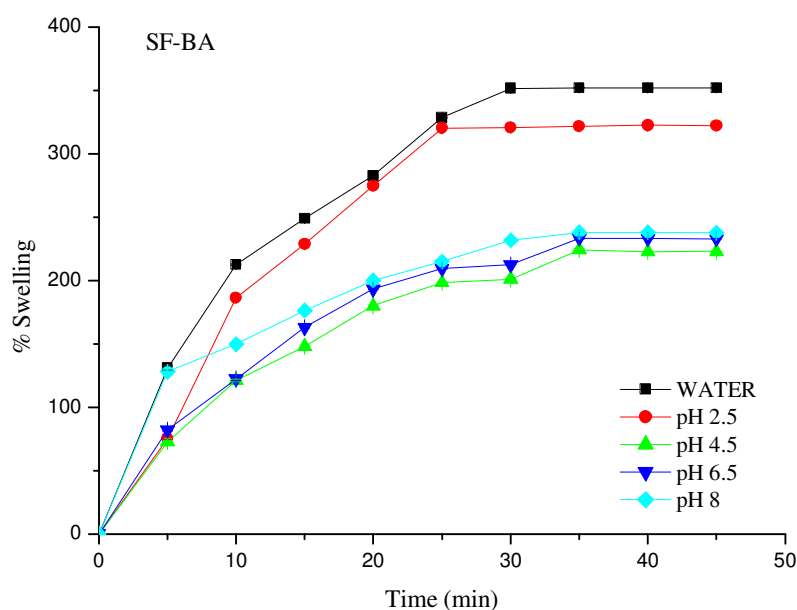


Figure 3.33 The swelling equilibrium times for hydrogel SF-BA

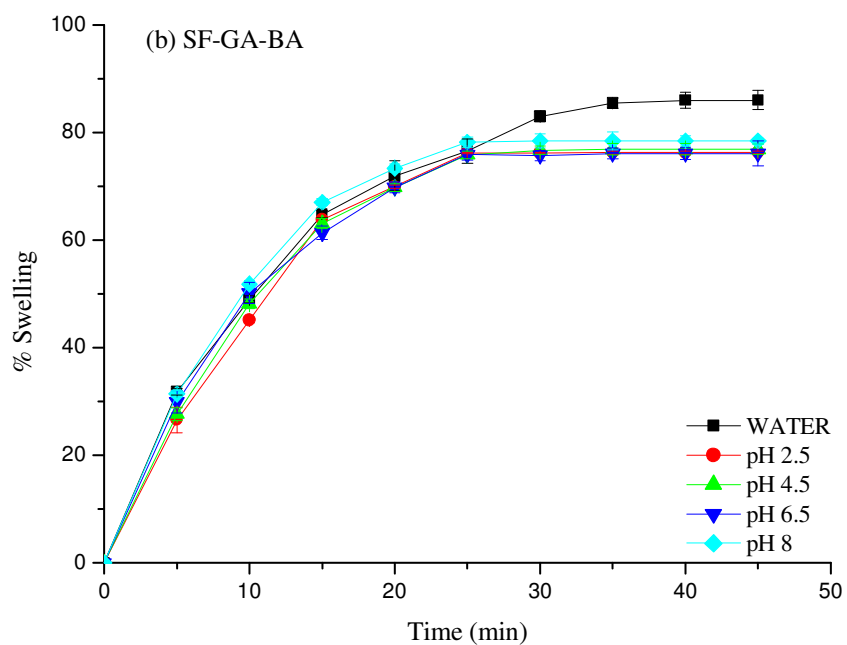
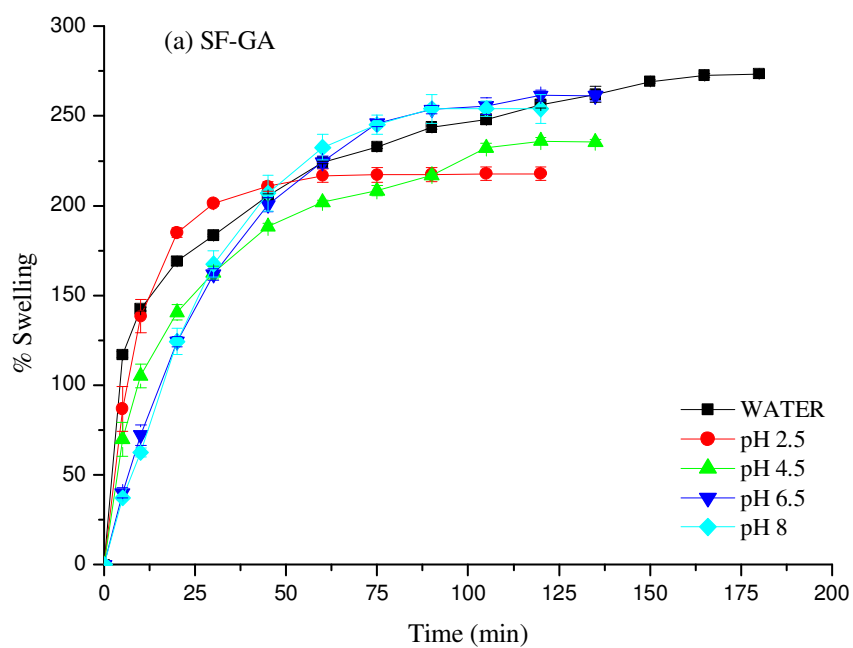


Figure 3.34 The swelling equilibrium times for the hydrogels (a) SF-GA and (b) SF-GA-BA

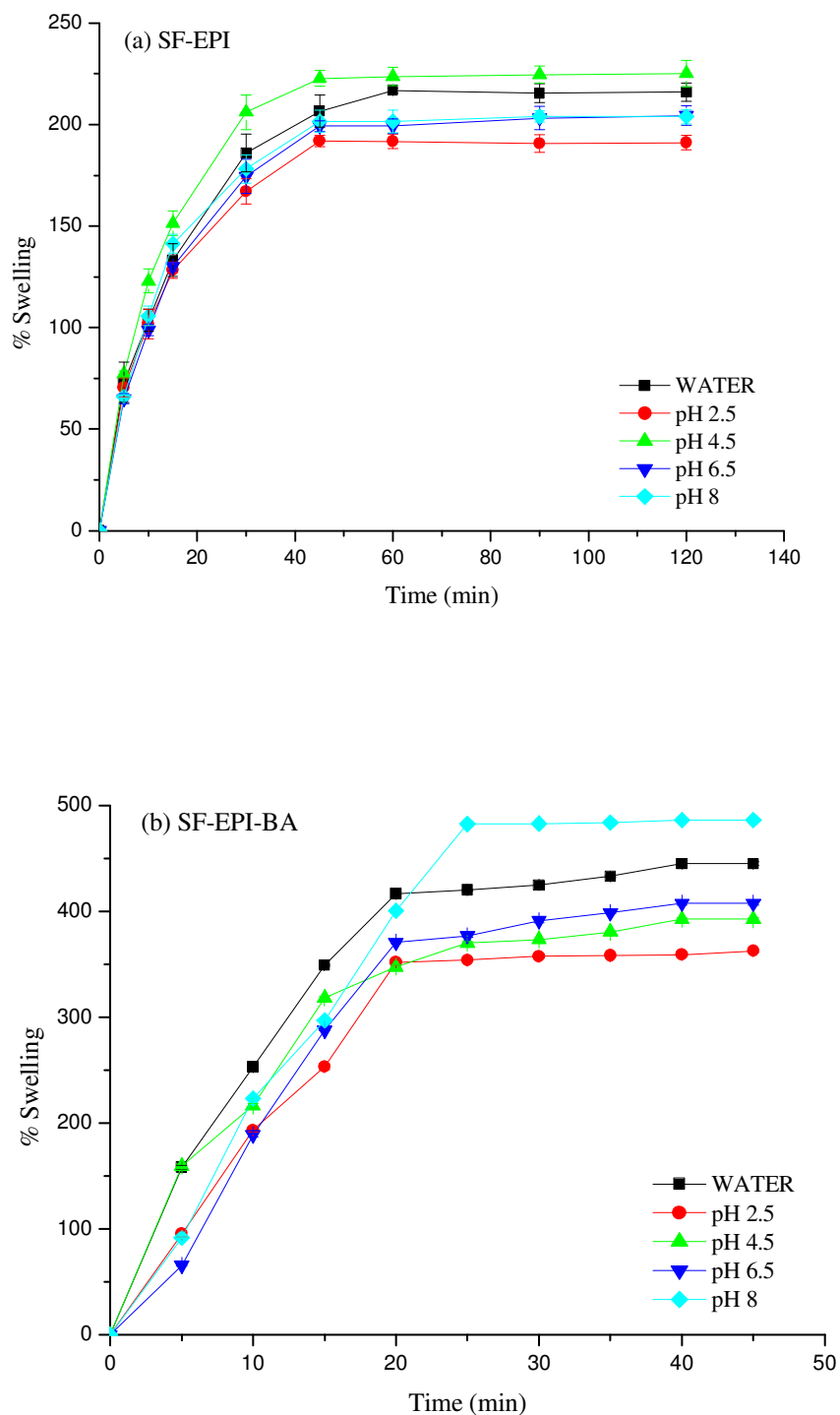


Figure 3.35 The swelling equilibrium times for the hydrogels (a) SF-EPI and (b) SF-EPI-BA

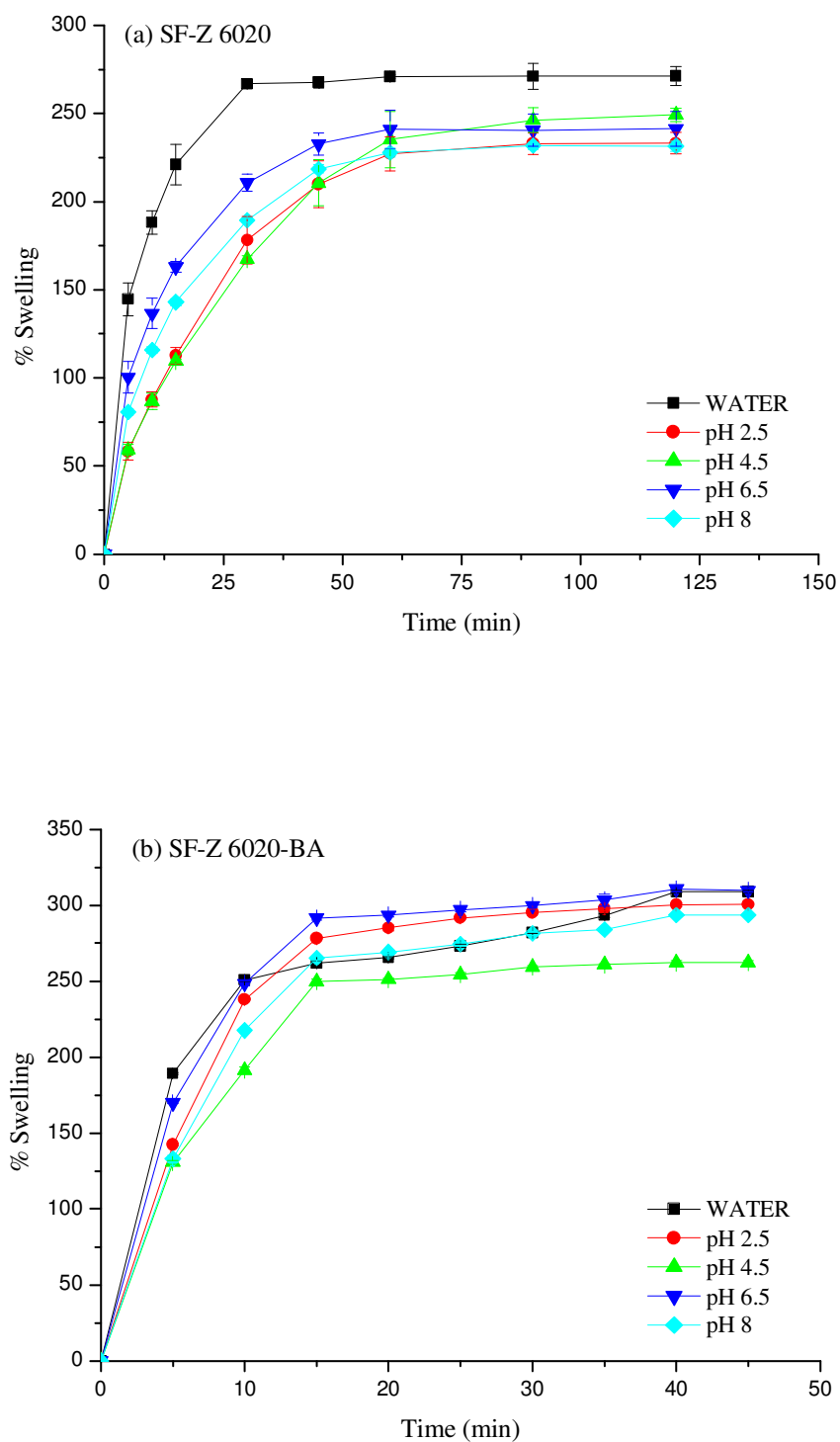


Figure 3.36 The swelling equilibrium times for the hydrogels (a) SF-Z 6020 and (b) SF-Z 6020-BA

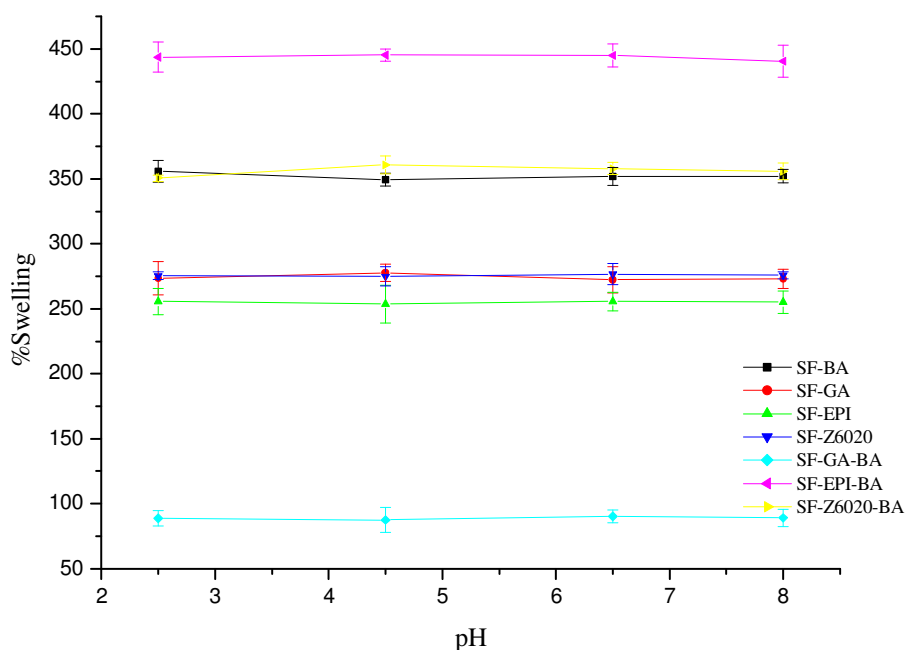


Figure 3.37 Swelling ratios for the hydrogels as a function of the pH (Swelling equilibrium time: 3h)

3.3 Solubility of Synthesized Powder Complexes

Crosslinking of polysaccharides is one of the most common ways to reduce the solubility of polysaccharides by formation of intermolecular bridges between the polysaccharide macromolecular chains.

The results of the solubility tests were represented in Table 3.6. All the cross-linked starches and the boron complexes of cross-linked starches exhibited lower solubility than potato starch. This may be attributed to the increase in chain length and in degree of the cross-linking as the granular structure of starch becomes more compact.

The decrease in solubility became greater as the concentration of the cross-linker was increased. The change in solubility was more significant for GA than EPI and Z-6020 when the amount of cross-linker was enhanced two times. Despite this, the amount of cross-linker was held minimum because of the toxicity of the cross-linking agents.

For all types of cross-linkers the boron complexes of cross-linked starches were less soluble than cross-linked starches. Even though, S-BA is the most soluble complex within all boron complexes of cross-linked starches because BA was insufficient for cross-linking of starch on its own relatively to the other cross-linking agents.

After cross-linking and complex formation the solubility of starch in water decreased between the ranges of 1%-12%. In terms of solubility it could be claimed that GA was the best appropriate cross-linker for the synthesis of boron complexes of cross-linked starches.

Table 3.6 Solubility of cross-linked starch powders and their boron complexes

Sample	Solubility (%)
Potato Starch	26.8
S-BA	25.7
CLS-GA	23.5
CLS-GA(2)	14.9
CLS-GA-BA	18.8
CLS-EPI	24.4
CLS-EPI(2)	23.2
CLS-EPI-BA	24.0
CLS-Z 6020	24.3
CLS-Z 6020(2)	20.4
CLS-Z 6020-BA	21.1

3.4 In vitro Antimicrobial Activity Tests of Synthesized Complexes

Antimicrobial activities of prepared Starch/PVA based hydrogels and starch based powder complexes were investigated. The results were given in Table 3.7 and Table 3.8, respectively.

Inhibition zone formations confirmed that SF-GA-BA and SF-GA hydrogels had antimicrobial activity. However, other hydrogels and all powder complexes had no antimicrobial activities against tested microorganisms.

The results denoted that SF-GA-BA and SF-GA showed moderate antibacterial activity and antifungal activity. SF-GA-BA indicated 14-mm inhibition zones against *Escherichia coli* (ATCC 25922) so as this result is comparable with standard antibiotic ER and 13-mm zones against *Staphylococcus aureus* (ATCC 25923), 12-mm zones against *Pseudomonas aeruginosa* (ATCC 27853) and 14-mm zones against *Candida albicans* (ATCC 10231) (Figure 3.38). Also, SF-GA indicated 6-mm inhibition zones against *Bacillus subtilis* (ATCC 11774) and 6-mm zones against *Candida albicans* (ATCC 10231). Thus, it can be seen from Figures 3.39-3.44, standard antibiotics showed different zones against tested organisms.

From the results obtained, it appears that the antibacterial action of the SF-GA-BA is more pronounced on Gram-negative than on Gram-positive bacteria. The reason of different sensitivity between Gram-positive and Gram-negative bacteria could be ascribed to the morphological differences between these microorganisms.

Table 3.7 Antimicrobial activities of synthesized hydrogels and standard antibiotics

Tested organisms	Zones of inhibition (mm)											
	Hydrogels (12 mg/9 mm disc)							Standard antibiotics (µg/6 mm paper disc)				
	SF-GA-BA	SF-BA	SF-GA	SF-EPI-BA	SF-EPI	SF-Z6020- BA	SF-Z6020	OFX 5	NET 30	ER 15	AMC 30	AFB 30
<i>Escherichia coli</i> ATCC 25922	14	–	–	–	–	–	–	28	26	14	20	NT
<i>Staphylococcus aureus</i> ATCC 25923	13	–	–	–	–	–	–	20	18	18	>30	NT
<i>Streptococcus pyogenes</i> ATCC 19615	–	–	–	–	–	–	–	22	>30	>30	28	NT
<i>Pseudomonas aeruginosa</i> ATCC 27853	12	–	–	–	–	–	–	16	22	R	R	NT
<i>Bacillus subtilis</i> ATCC 11774	–	–	6	–	–	–	–	24	>30	>30	24	NT
<i>Candida albicans</i> ATCC 10231	14	–	6	–	–	–	–	NT	NT	NT	NT	13

OFX: ofloxacin; NET: netilmycin; ER: erythromycin; AMC: amoxycillin /clavulanic acid; AFB: amphotericin; B; NT: not tested; R: resistance; –: not active

Table 3.8 Antimicrobial activities of synthesized powder complexes and standard antibiotics

Tested organisms	Zones of inhibition (mm)											
	Powder complexes (100 µg / 9 mm disc)							Standard antibiotics (µg/6 mm paper disc)				
	CLS-GA-BA	S-BA	CLS-GA	CLS-EPI-BA	CLS-EPI	CLS-Z6020-BA	CLS-Z6020	OFX 5	NET 30	ER 15	AMC 30	AFB 30
<i>Escherichia coli</i> ATCC 25922	–	–	–	–	–	–	–	28	26	14	20	NT
<i>Staphylococcus aureus</i> ATCC 25923	–	–	–	–	–	–	–	20	18	18	>30	NT
<i>Streptococcus pyogenes</i> ATCC 19615	–	–	–	–	–	–	–	22	>30	>30	28	NT
<i>Pseudomonas aeruginosa</i> ATCC 27853	–	–	–	–	–	–	–	16	22	R	R	NT
<i>Bacillus subtilis</i> ATCC 11774	–	–	–	–	–	–	–	24	>30	>30	24	NT
<i>Candida albicans</i> ATCC 10231	–	–	–	–	–	–	–	NT	NT	NT	NT	13

OFX: ofloxacin; NET: netilmycin; ER: erythromycin; AMC: amoxycillin /clavulanic acid; AFB: amphotericin-B; NT: not tested; R: resistance; –: not active

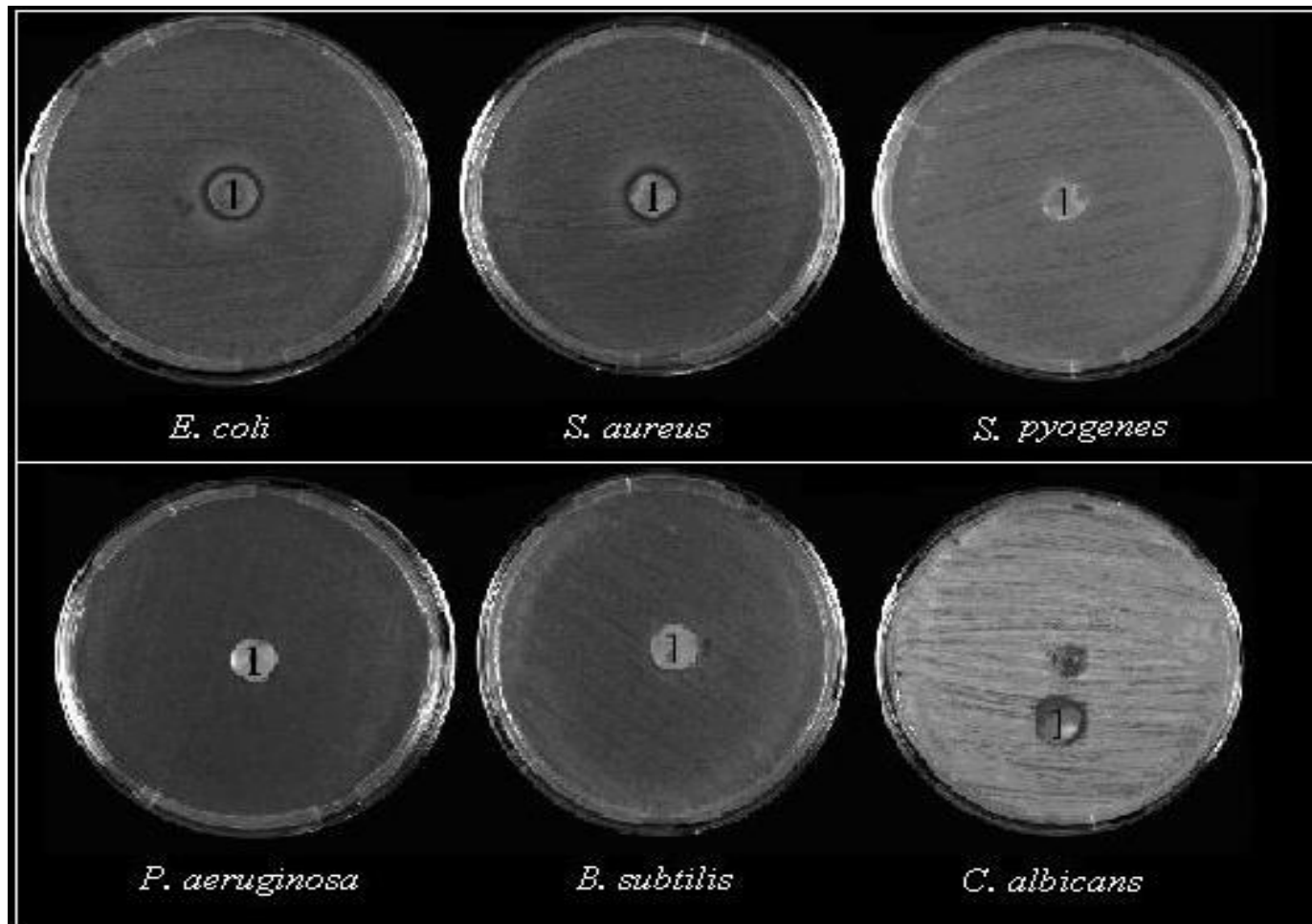


Figure 3.38 The antimicrobial activities of SF-GA-BA on *E. coli* [ATCC 25922], *S. Aureus* [ATCC 25923], *S. pyogenes* [ATCC 19615], *P. aeruginosa* [ATCC 27853], *B. subtilis* [ATCC 11774], and *Candida albicans* [ATCC 10231]

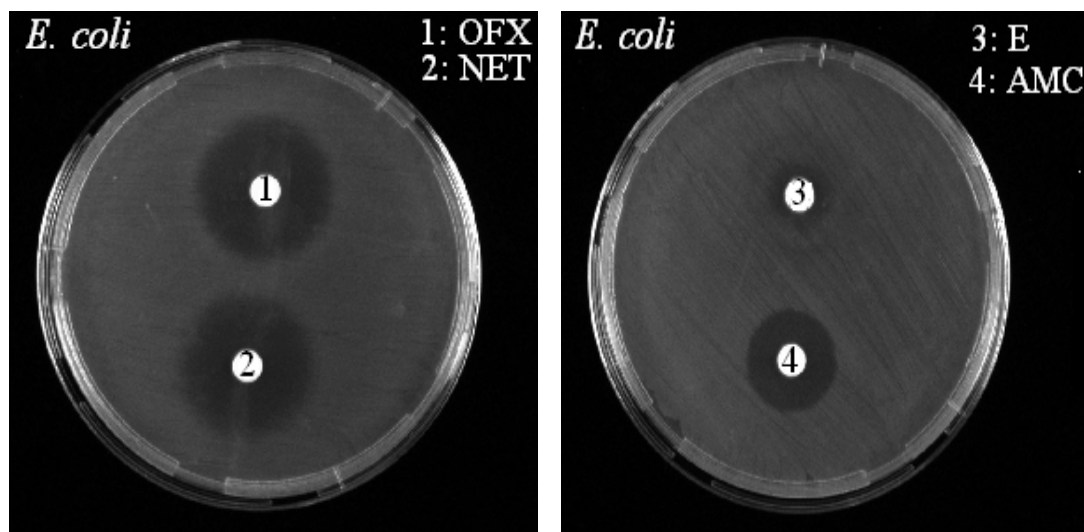


Figure 3.39 Antimicrobial activities of various antibiotics on growth of *E. coli* ATCC 25922
1: Ofloxacin 2: Netilmycin 3: Erythromycin 4: Amoxycillin/clavulanic acid

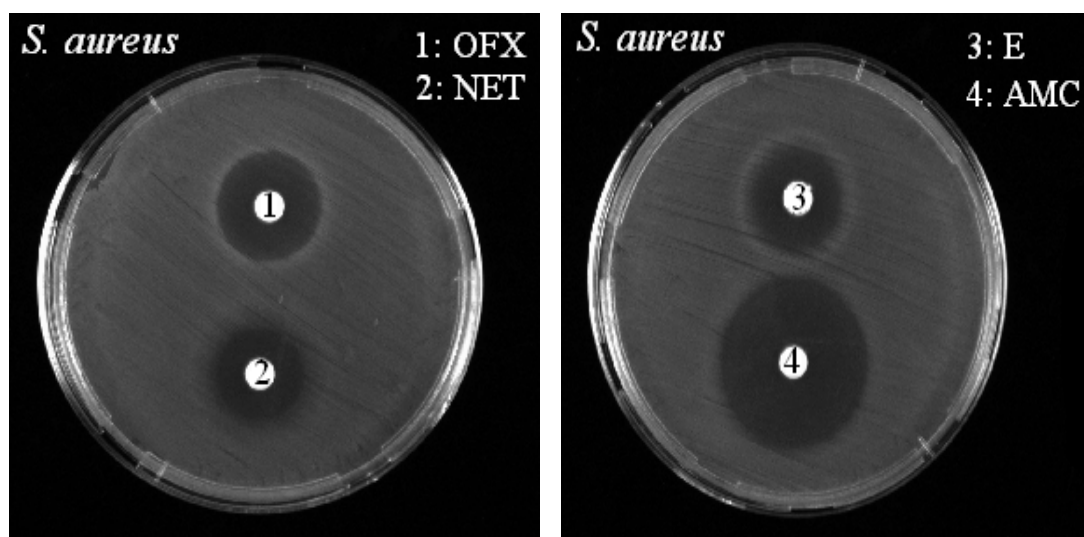


Figure 3.40 Antimicrobial activities of various antibiotics on growth of *S. aureus* ATCC 25923
1: Ofloxacin 2: Netilmycin 3: Erythromycin 4: Amoxycillin/clavulanic acid

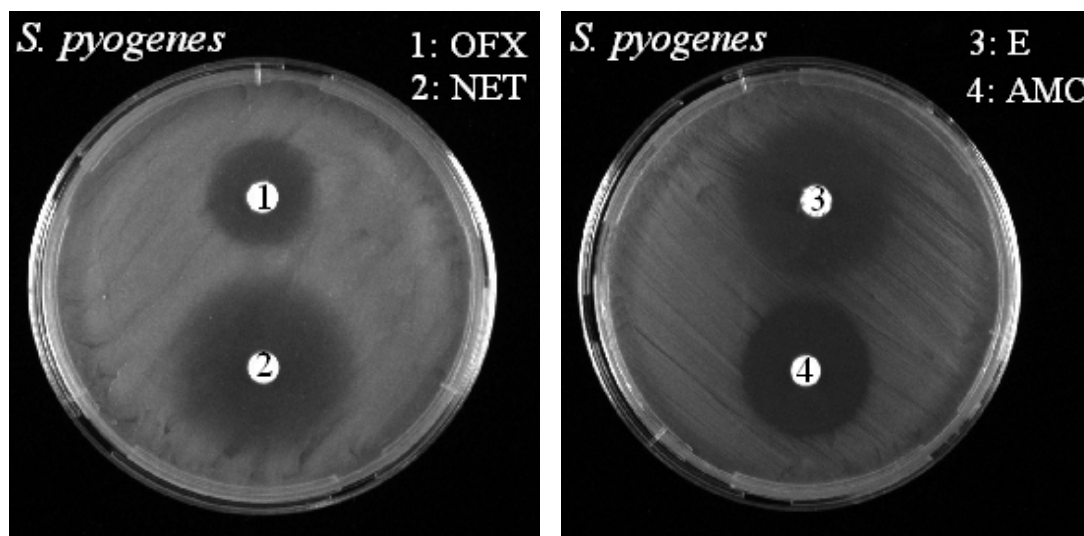


Figure 3.41 Antimicrobial activities of various antibiotics on growth of *S. pyogenes* ATCC 19615
1: Ofloxacin 2: Netilmycin 3: Erythromycin 4: Amoxicillin/clavulanic acid

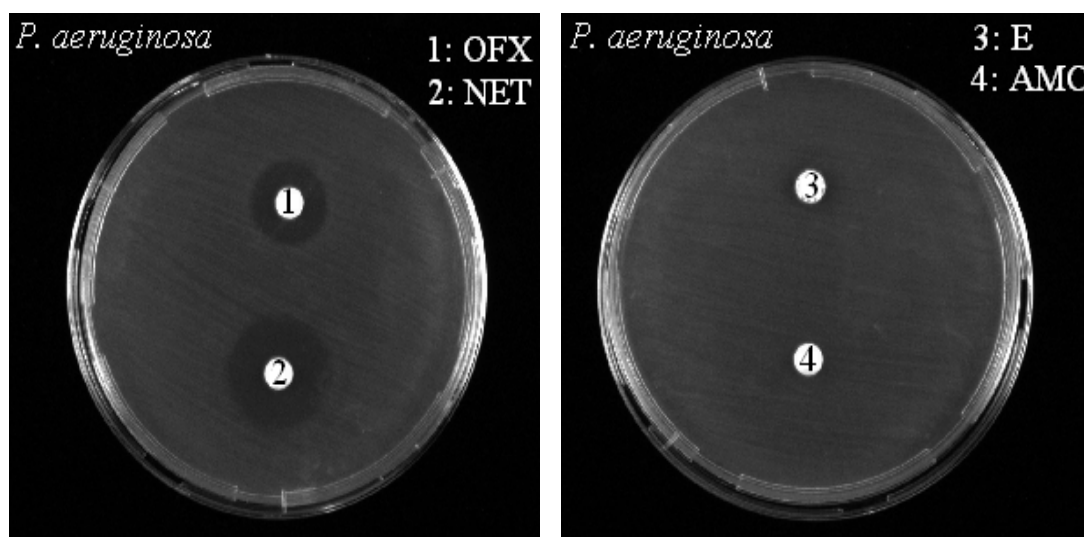


Figure 3.42 Antimicrobial activities of various antibiotics on growth of *P. aeruginosa* ATCC 27853
1: Ofloxacin 2: Netilmycin 3: Erythromycin 4: Amoxicillin/clavulanic acid

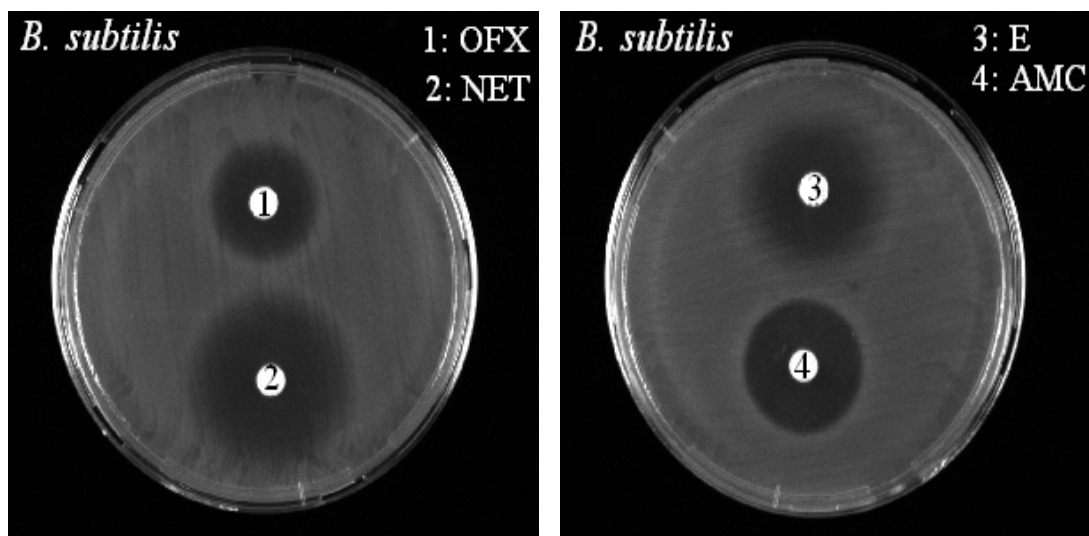


Figure 3.43 Antimicrobial activities of various antibiotics on growth of *B. subtilis* ATCC 11774
1: Ofloxacin 2: Netilmycin 3: Erythromycin 4: Amoxicillin/clavulanic acid

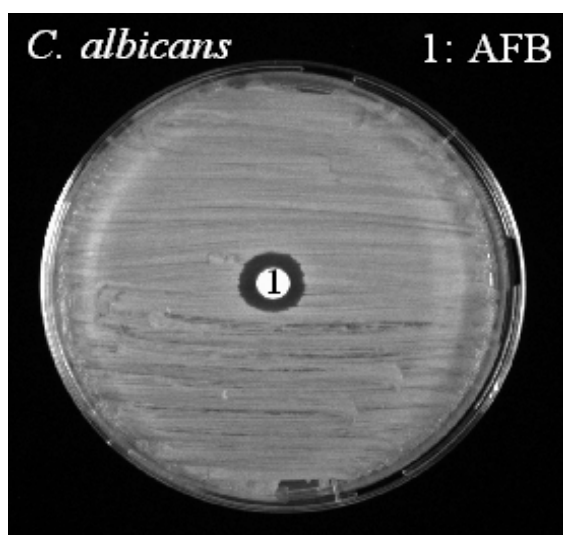


Figure 3.44 The antifungal activity of amphotericin B on *C. albicans* ATCC 10231

CHAPTER FOUR

CONCLUSION

4.1 Conclusion

This study was consisted of three basic stages; preparation and characterization of boron-starch complexes and investigation of their antimicrobial activities.

Boron complexes of starch which were unmodified or modified by cross-linking reactions were prepared either in powder or hydrogel forms. PVA was used to provide a stable support medium for starch hydrogels. Three different cross-linkers; GA, EPI and Z-6020, were used to obtain cross-linked starches.

FTIR, TGA, SEM and XRD methods were used and degree of solubility, swelling and TS tests were performed in order to clarify the structures and some properties of synthesized complexes. Also *in vitro* antimicrobial activities of prepared complexes were investigated for five different bacterial cultures and one fungus.

According to XRD patterns, a semi-crystalline compound, starch had just one strong diffraction peak at approximately $2\theta=17.0^\circ$ and PVA had two peaks at $2\theta=18.9^\circ$ and 38.9° , the former being most intense. The X-ray diffractograms of hydrogels revealed amorphous compounds, with intense peaks at approximately $2\theta=20.0^\circ$ indicating that the crystallinity imparted in the hydrogels were mainly due to crystallinity of PVA. There appeared an increase in overall intensities of the XRD reflections with cross-linking due to gelatinization of starch during synthesis of the hydrogels. Gelatinization was much for SF-GA-BA than SF-GA. The same result could not be seen for SF-EPI-BA and SF-Z 6020-BA indicating the lower gelatinization degree in these complexes compared to SF-EPI and SF-Z 6020.

Otherwise, with increasing degree of cross-linking the crystallinity capacities were reduced and the intensities of sharp peaks of diffraction occurred in the spectrogram of starch were diminished for all boron containing powder complexes except CLS-Z6020-BA. In this case, cross-linking is claimed to be more effective in boron complexes of starches which were cross-linked with GA and EPI than Z-6020. Additionally, the crystal area of the starch was not greatly affected when crosslinking agents were used alone in the synthesis without using BA. But the degree of cross-linking was enhanced for boron complexes of cross-linked starches, resulting a reduction in crystallinity. No single crystal peaks of BA could be identified in boron containing complexes, suggesting that all BA was used in the synthesis procedure.

When the FTIR spectrums of raw materials were evaluated, some bands related to hydrogen-bonded hydroxyl groups, C-H stretching, C-H bending, C-O and C-C stretchings were seen approximately in the same regions for both starch and PVA.

There were no bands in the FTIR spectrum of the hydrogels that highly differ from the bands corresponding to their individual components. There were just small shifts to higher or lower frequencies and shape or intensity changes in these bands. So this reveals the functional groups in the structure of starch and PVA did not almost change with cross-linking and complex formation.

Changes in FTIR bands of powder complexes also showed similar changes like hydrogels.

The absence of bands in the range of $1740-1720\text{ cm}^{-1}$ for all GA containing complexes indicated absence of aldehydic groups so it could be claimed that all the aldehydic groups of the GA has been used for crosslinking.

In the case of cross-linking with Z-6020; N-H stretchings, C-N stretching of amines, $\text{CH}_2\text{-N}$ stretchings, NH_2 deformations, Si-O-Si asymmetric stretchings, Si-C, Si-O-C and Si-OH bands were also observed in addition to other common bands.

Appearance of broad asymmetric stretchings bands of Si-O-Si showed that siloxane polymers are formed. Thus the silanes are first hydrolysed and then condensed to oligomers.

Characteristic bands of BA such as B-O stretching, O-B-O bendings and B-O deformation bands could not be distinguished from the other bands of raw materials in the synthesized hydrogels but for powder complexes, these bands were rather changed in shape and some of the bands were disappeared or overlapped with some other bands related to starch in the synthesized powder complexes. From this it can be inferred that BA has been completely used in the complex formation.

The TGA thermograms and derivative thermogravimetric (DTG) curves of synthesized complexes showed that starch, cross-linked starches and boron complexes of starches in the powder form exhibited two-step decompositions while PVA and all synthesized hydrogels were exhibiting three-step thermal decomposition. The first step of mass losses could be attributed to evaporation of adsorbed water for all samples. The other steps were due to thermal decomposition stages of the products. Evaporation of adsorbed water was carried out between 110-125 °C with 3-9% and 46-63 °C with maximum 12% mass losses for hydrogels and powder complexes, respectively where thermal decompositions were realized above 300 °C and 400 °C with an average 58% and 20% mass losses, respectively for second and third steps of degradation for hydrogels and between 276-299 °C with approximately 65-80% mass losses for powder complexes.

It is concluded that the cross-linking of starch/PVA hydrogels lead to the formation of more thermally stable copolymers than starch and PVA. The use of BA in the synthesis of the hydrogels improves the thermal stability of starch and PVA more than cross-linked starch/PVA hydrogels. Unlike hydrogels thermal stability of starch was decreased for both cross-linked starches and boron complexes of starch after modification processes. The amount of cross-linking agents used in the synthesis of powder complexes was less than hydrogels so the result was acceptable for powder complexes.

When the morphology of the raw materials used in this study were investigated; the potato starch exhibited good sphericity and a smooth surface besides PVA has a rough surface. Also, BA was observed like spherical shaped particles with a diameter of approximately 400 μm .

All cross-linked starch/PVA hydrogels revealed an irregular morphology. The SEM analysis results showed that the morphology of starch was changed when starch was blended with PVA in the synthesized hydrogels as already described and demonstrated with XRD analysis. There were holes and pores inside the materials. Irregular and incompact structure was observed for SF-BA. This situation showed us that, BA by itself was insufficient for the cross-linking of starch with PVA compared to other cross-linking agents.

According to SEM images of the boron-starch powder complexes, it could be claimed that when starch was treated with BA or EPI, big aggregates were formed. Besides glutaraldehyde did not give any aggregates and Z-6020 gave just small ones. When BA and cross-linkers were used together in the synthesis the aggregates of S-BA were dissociated into smaller particles. After being cross-linked, potato starch granules remain smooth and similar to native starch granules in morphology when viewed under SEM, suggesting that the modification does not cause any detectable morphological change.

The mechanical properties of starch/PVA hydrogels were measured and t-test was performed to compare the experimental data and it was found that the difference was not statistically significant ($p > 0.05$) among the TS, E and ϵ % of the hydrogels.

The alteration of E expressed a tendency similar to that of the TS. The average value of TS was found 44.5 MPa for synthesized hydrogels which is comparable with the failure strength of skin with 34 MPa (Bhat, 2002).

Wide variations were found in TS values ranging from approximately 10 to 50 MPa for different types of hydrogels or chemically cross-linked blends used for biomedical applications (Elvira, Mano, San Roman & Reis, 2002, Pal et al., 2006b, Dean, Do, Petinakis & Yu, 2008, Costa-Júnior, Barbosa-Stancioli, Mansur, A.P., Vasconcelos & Mansur, H.S., 2009).

So, it could be claimed that all synthesized hydrogels in this study had suitable mechanical properties which could be offered for potential use in biomedical applications.

The results of equilibrium swelling times of the hydrogels at ultra pure water and citric acid-phosphate solutions of definite pH values were investigated and the results showed that swelling equilibrium times were shorter for the boron containing hydrogels compared to just cross-linked hydrogels. Swelling percentages were calculated between 200-300% for cross-linked hydrogels and 300-500% for boron complexes of starch/PVA hydrogels except SF-GA-BA with a minimum swelling ratio of 86%. Therefore, it could be suggested that the major cross-linking was occurred in SF-GA-BA, because it was known that cross-linking causes the granules to become compact and absorb less water than the native starch so decreased the swelling power.

When BA was used for complex formation together with EPI and Z-6020 or just by itself, proper compact structure which was seen in SF-GA-BA or in cross-linked starch/PVA hydrogels could not be achieved, thereby swelling ratios were increased.

During the pH dependent swelling experiments, the hydrogels were swollen at water with certain swelling percentages until they reached equilibrium. When the same samples were immersed in solutions of different pH values and leaved in each solution for maximum three hours no remarkable change was observed in the swelling ratios denoting that the swelling behavior of the membranes were essentially independent of pH. As starch and PVA contained no ionizable functional groups, this result was compatible with the expected one.

Cross-linking treatment was performed in this thesis study during the synthesis of the complexes in order to reduce the solubility of starches. As the granular structure of starch becomes more compact, all the cross-linked starch powders and the boron complexes of cross-linked starches in the powder form exhibited lower solubility than potato starch. The decrease in solubility became greater as the concentration of the cross-linker was increased. For all types of cross-linkers, the cross-linked starch boron complexes were less soluble than cross-linked starches.

Even though, S-BA is the most soluble complex within all boron complexes of cross-linked starches because BA was insufficient for cross-linking of starch on its own relatively to the other cross-linking agents. After cross-linking and complex formation the solubility of starch in water decreased between the ranges of 1%-12%. In terms of solubility it could be claimed that GA was the best appropriate cross-linker for the synthesis of boron complexes of cross-linked starches.

Antimicrobial activities of prepared starch/PVA based hydrogels and starch based powder complexes were investigated. Inhibition zone formations confirmed that SF-GA-BA and SF-GA hydrogels showed moderate antibacterial and antifungal activities. However, other hydrogels and all powder complexes had no antimicrobial activities against tested microorganisms.

From the results obtained, it appears that the antibacterial action of the SF-GA-BA is better than SF-GA and more pronounced on Gram-negative than on Gram-positive bacteria. The reason of different sensitivity between these bacteria could be ascribed to the morphological differences between these microorganisms.

As a conclusion; considering all results obtained in this thesis study, it could be that the boron complexes of starch/PVA hydrogels cross-linked with GA might be used in contact lenses, artificial skin and corneas, superficial burns or in the treatment of chronic wounds containing light exudates due to showing antimicrobial activities.

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